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NEWS	10	DEC 01	CAS REGISTRY updated with new ambiguity codes
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NEWS	12	DEC 14	WPIDS/WPINDEX/WPIX manual codes updated
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NEWS	15	DEC 18	CA/CAplus patent kind codes updated
NEWS	16	DEC 18	MARPAT to CA/CAplus accession number crossover limit increased to 50,000
NEWS	17	DEC 18	MEDLINE updated in preparation for 2007 reload
NEWS	18	DEC 27	CA/CAplus enhanced with more pre-1907 records
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=> s recombinant Escherichia coli
L1        8476 RECOMBINANT ESCHERICHIA COLI

=> s l1 and heat-killed
L2           0 L1 AND HEAT-KILLED

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L3           0 L1 AND MODIFIED ALLERGEN

=> s Escherichia coli
L4     1162694 ESCHERICHIA COLI

=> s l4 and recombinant allergen
L5         462 L4 AND RECOMBINANT ALLERGEN

=> s l5 and modified
L6          19 L5 AND MODIFIED

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L7           0 L6 AND HEAT-KILLED

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L8            9 DUP REMOVE L6 (10 DUPLICATES REMOVED)

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L8   ANSWER 1 OF 9     MEDLINE on STN
2006548078.    PubMed ID: 16956374.   Ca2+-binding allergens from olive pollen
     exhibit biochemical and immunological activity when expressed in stable
     transgenic Arabidopsis. Ledesma Amalia; Moral Veronica; Villalba Mayte;
     Salinas Julio; Rodriguez Rosalia. (Dpto. Bioquimica y Biologia Molecular
     I, Universidad Complutense, Madrid, Spain. ) The FEBS journal, (2006 Oct)
     Vol. 273, No. 19, pp. 4425-34.  Electronic Publication: 2006-09-05.
     Journal code: 101229646. ISSN: 1742-464X. Pub. country: England: United
     Kingdom. Language: English.
AB   Employing transgenic plants as alternative systems to the conventional
     Escherichia coli, Pichia pastoris or baculovirus hosts
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to produce **recombinant allergens** may offer the possibility of having available edible vaccines in the near future. In this study, two EF-hand-type Ca2+-binding allergens from olive pollen, Ole e 3 and Ole e 8, were produced in transgenic Arabidopsis thaliana plants. The corresponding cDNAs, under the control of the constitutive CaMV 35S promoter, were stably incorporated into the Arabidopsis genome and encoded recombinant proteins, AtOle e 3 and AtOle e 8, which exhibited the molecular properties (i.e. MS analyses and CD spectra) of their olive and/or E. coli counterparts. Calcium-binding assays, which were carried out to assess the biochemical activity of AtOle e 3 and AtOle e 8, gave positive results. In addition, their mobilities on SDS/PAGE were according to the conformational changes derived from their Ca2+-binding capability. The immunological behaviour of Arabidopsis-expressed proteins was equivalent to that of the natural- and/or E. coli-derived allergens, as shown by their ability to bind allergen-specific rabbit IgG antiserum and IgE from sensitized patients. These results indicate that transgenic plants constitute a valid alternative to obtain allergens with structural and immunological integrity not only for scaling up production, but also to develop new kind of vaccines for human utilization.

L8 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1

2006223169. PubMed ID: 16630158. Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of Brown Shrimp, Penaeus aztecus. Reese G; Schicktanz S; Lauer I; Randow S; Luttkopf D; Vogel L; Lehrer S B; Vieths S. (Paul-Ehrlich-Institut, Department of Allergology, Langen, Germany.. reege@pei.pe) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Apr) Vol. 36, No. 4, pp. 517-24. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: **Recombinant allergens** are considered the basis for new diagnostic approaches and development of novel strategies of allergen-specific immunotherapy. As Pen a 1 from brown shrimp Penaeus aztecus is the only major allergen of shrimp and binds up to 75% of all shrimp-specific IgE antibodies this molecule may be an excellent model for the usage of allergens with reduced IgE antibody-binding capacity for specific immunotherapy. AIM: The aim was to clone, express and characterize a full-length recombinant Pen a 1 molecule and compare it with natural Pen a 1 in regard to structural and immunological parameters such as IgE antibody capacity and ability to induce IgE-mediated mediator release. METHODS: Total RNA was isolated from P. aztecus and a rapid amplification of cDNA ends (5' RACE) was performed to obtain full-length cDNA coding for Pen a 1. Using a gene-specific primer, PCR was performed and full-length cDNA was cloned and sequenced. Recombinant His-tagged Pen a 1 was isolated from **Escherichia coli** under native conditions by immobilized metal affinity chromatography. Secondary structure of natural and recombinant Pen a 1 was compared by circular dichroism (CD) spectroscopy, and the IgE antibody-binding capacity evaluated by RAST. The allergenic potency was tested by the capability of natural and recombinant Pen a 1 to induce mediator release in a murine and human in vitro model of IgE-mediated type I allergy. RESULTS: The deduced amino-acid sequence was 284 residues long and amino-acid sequence identities with allergenic and non-allergenic tropomyosins ranged from 80% to 99% and 51% to 58%, respectively. The analysis of the secondary structure of natural and recombinant Pen a 1 by CD spectroscopic analysis showed that both nPen a 1 and rPen a 1 had alpha-helical conformation that is typical for tropomyosin. The IgE antibody binding capacities of nPen a 1 and r Pen a1 were found to be essentially identical by RAST. The mediator release experiments using both wild-type and humanized rat basophilic leukaemia 30/25 cells showed that rPen a 1 and nPen a 1 induced a similar level of mast cell activation. CONCLUSIONS: Recombinant Pen a 1 and natural Pen a 1 are structurally and immunologically identical and

rPen a 1 may be used as the basis for component-resolved diagnosis and the generation of **modified** shrimp tropomyosin for allergen-specific immunotherapy. The results of the animal studies indicate that C3H/HeJ mice that were sensitized with shrimp extract in combination with cholera toxin as adjuvant may be a suitable model to study shrimp allergy.

L8 ANSWER 3 OF 9 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

2006254921 EMBASE Recombinant expression systems for allergen vaccines. Singh M.B.; Bhalla P.L.. M.B. Singh, Institute of Land and Food Resources, The University of Melbourne, Parkville, Vic. 3010, Australia. mohan@unimelb.edu.au. Inflammation and Allergy - Drug Targets Vol. 5, No. 1, pp. 53-59 2006.
Refs: 60.
ISSN: 1871-5281. Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 20060615. Last Updated on STN: 20060615

AB Allergen immunotherapy of future is likely to be based on allergy vaccines that contain engineered allergens **modified** to abolish or substantially reduce their IgE-binding activity in order to remove the risk of unwanted anaphylactic responses. The development of efficient systems for the production of **recombinant allergens** in sufficient quantities is requirement for establishing use of engineered allergens as components of allergy vaccines. This review outlines relative advantages and disadvantages of various heterologous systems for production of **recombinant allergens**. Microbial systems are most convenient and cost effective platforms for the production of **recombinant allergens**. However, lack of post-translational processing implies that some allergens have to be expressed in eukaryotic systems for proper folding and post-translational modifications such as glycosylation. Yeast systems can yield high levels of **recombinant allergens** but often are associated with hyper- glycosylation problems. Mammalian cell culture systems offer suitable post -translational modifications but are nearly hundred fold more expensive than microbial systems. The use of plants as bio-factories for production of **recombinant allergens** is emerging as a very attractive option as plants-based production system offer several advantages over other expression systems such as post translational processing of proteins, low production costs, scale up ability and enhanced safety due to absence of animal or human pathogens. .COPYRGT. 2006 Bentham Science Publishers Ltd.

L8 ANSWER 4 OF 9 MEDLINE on STN

2004221551. PubMed ID: 15119037. Expression of allergens in E. coli and plants--benefits and drawbacks. Breiteneder Heimo; Wagner Birgit. (Dept. of Pathophysiology, University of Vienna, Wahringer Gurtel 18-20, A-1090 Vienna.) Arbeiten aus dem Paul-Ehrlich-Institut (Bundesamt fur Sera und Impfstoffe) zu Frankfurt a.M, (2003) No. 94, pp. 178-87. Ref: 51. Journal code: 8912864. ISSN: 0936-8671. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB **Recombinant allergens** are quickly becoming the reagents of choice for diagnosis and therapy of type I allergic diseases. Consequently, the different methods for the production of recombinant proteins that are available today are of great interest to allergologists. Without doubt, bacterial expression will continue to play a pivotal role. In addition, plant-based expression systems will be needed to overcome problems inherent in the E. coli systems and to allow the production of glycoallergens or allergens of more complex folding.

L8 ANSWER 5 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2003:74387 The Genuine Article (R) Number: 632HU. Art v 1, the major allergen

of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. Himly M; Jahn-Schmid B; Dedic A; Kelemen P; Wopfner N; Altmann F; van Ree R; Briza P; Richter K; Ebner C; Ferreira F (Reprint). Salzburg Univ, Inst F Genet U Allg Biol, Hellbrunnerstr 34, A-5020 Salzburg, Austria (Reprint); Salzburg Univ, Inst Genet & Gen Biol, A-5020 Salzburg, Austria; Univ Vienna, Inst Pathophysiol, A-1090 Vienna, Austria; Univ Agr Vienna, Inst Chem, A-1190 Vienna, Austria; Sanquin Res CLB, Dept Immunopathol, NL-1066 CX Amsterdam, Netherlands. fatima.ferreira @mh.sbg.ac.at. FASEB JOURNAL (NOV 2002) Vol. 16, No. 13, pp. 106-+. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In late summer, pollen grains originating from Compositae weeds (e.g., mugwort, ragweed) are a major source of allergens worldwide. Here, we report the isolation of a cDNA clone coding for Art v 1, the major allergen of mugwort pollen. Sequence analysis showed that Art v 1 is a secreted allergen with an N-terminal cysteine-rich domain homologous to plant defensins and a C-terminal proline-rich region containing several (Ser/Ala)(Pro)(2-4) repeats. Structural analysis showed that some of the proline residues in the C-terminal domain of Art v 1 are posttranslationally **modified** by hydroxylation and O-glycosylation. The O-glycans are composed of 3 galactoses and 9-16 arabinoses linked to a hydroxyproline and represent a new type of plant O-glycan. A 3-D structural model of Art v 1 was generated showing a characteristic "head and tail" structure. Evaluation of the antibody binding properties of natural and recombinant Art v 1 produced in **Escherichia coli** revealed the involvement of the defensin fold and posttranslational modifications in the formation of epitopes recognized by IgE antibodies from allergic patients. However, posttranslational modifications did not influence T-cell recognition. Thus, recombinant nonglycosylated Art v 1 is a good starting template for engineering hypoallergenic vaccines for weed-pollen therapy.

L8 ANSWER 6 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2000:267299 The Genuine Article (R) Number: 297YX. **Recombinant allergens**: application to diagnostic and therapeutic perspectives. Pauli G (Reprint); Deviller P. Hop Univ Strasbourg, Serv Pneumol, BP 426, F-67091 Strasbourg, France (Reprint); Hop Univ Strasbourg, Serv Pneumol, F-67091 Strasbourg, France. REVUE DES MALADIES RESPIRATOIRES (FEB 2000) Vol. 17, No. 1BIS, pp. 293-303. ISSN: 0761-8425. Publisher: MASSON EDITEUR , 120 BLVD SAINT-GERMAIN, 75280 PARIS 06, FRANCE. Language: French.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Techniques of generic engineering applied to allergens have enabled the production of **recombinant allergens**. The validation of **recombinant allergens** implies that their immunological activity and their identity with natural allergens might be confirmed by in vitro and in vivo techniques carried out on a sufficiently large number of allergic subjects. Currently available results for the principal pneumoallergens are reported. Thus the work of validating **recombinant allergen** BeTv1 has been confirmed by in vitro tests and also by skin tests and nasal and bronchial provocation tests. The association of four **recombinant allergens** of phleole has enabled the detection in vitro of sensitisation to germinated pollens in 94.5% of patients. For mites the validity of group 2 **recombinant allergens** has been confirmed. A system enabling the expression of glycosylation of recombinant proteins was necessary to validate recombinant proteins in group 1 allergens. The **recombinant allergen** Blot5 is recognised as bring effective in the detection of sensitization to Blomia tropicalis, a domestic allergen in sub tropical countries. The **recombinant allergens** Bla g 4 and Bla g 5 have been tested in vitro and in

vivo and reactions were positive in nearly 50% of subjects sensitive to cockroaches. The recombinant Asp f 1 has been tested in subjects suffering from allergic bronchopulmonary aspergillosis and is positive in 60-85% of cases.

Some studies are available for **recombinant allergens** of certain animal antigens (Equ c 1, Bos d 2). The consequences of clarifying **recombinant allergens** are then analysed : obtaining better standardised allergens for diagnostic tests, studying the spectrum of specificities of IgE induced by an allergen, the quantification of specific IgE, a better approach to mixed allergies with the help of **recombinant allergens** of the principal mixed allergens. Some recent progress has led to the production of **modified recombinant allergens** : the synthesis of recombinant polypeptides corresponding to T epitopes, the production of isoform **recombinant allergens** with reduced allergenic activity, the production of **recombinant allergens** of **modified** allergenic molecules by directed mutations and the production of recombinant fragments of allergenic molecules. The use of **modified recombinant allergens** is a way of permitting research which would, in the future, lead to new modalities of specific immunotherapy.

L8 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 2

1999242424. PubMed ID: 10224369. The importance of **recombinant allergens** for diagnosis and therapy of IgE-mediated allergies. Kraft D; Ferreira F; Vrtala S; Breiteneder H; Ebner C; Valenta R; Susani M; Breitenbach M; Scheiner O. (Institute of General and Experimental Pathology, University of Vienna, Austria.) International archives of allergy and immunology, (1999 Feb-Apr) Vol. 118, No. 2-4, pp. 171-6. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB In the past 10 years, a considerable number of cDNAs coding for allergens have been isolated and expressed. Intensive investigations showed that **recombinant allergens** and their respective natural counterparts possess comparable properties with respect to structure, function and interaction with the immune system. Recent studies documented that in vitro as well as in vivo diagnosis of IgE-mediated allergic diseases can be successfully improved by the application of **recombinant allergens**. In addition, new strategies for a safer specific immunotherapy (SIT) have been developed based on the knowledge of the primary structures of allergens. Naturally occurring isoforms of allergens as well as **recombinant allergens** with **modified** amino acid sequences show very low IgE binding capacity but strong T cell-stimulatory activity and represent possible candidates. In case of Bet v 1, the major birch pollen allergen, isoforms d, g and l and a Bet v 1a mutant, produced by site-directed mutagenesis resulting in 6 amino acid exchanges, fulfilled the above mentioned criteria. In a third approach, two adjacent peptides covering the entire Bet v 1a sequence were produced in an **Escherichia coli** expression system. These peptides contained most of the relevant T cell epitopes, but lost their IgE binding capacity and, thus, their ability to activate mast cells and basophils of sensitized patients. Our results suggest that allergen variants (isoforms, mutants, T cell epitope-containing peptides) may be used as 'hypoallergenic agents' in SIT.

L8 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:98094 The Genuine Article (R) Number: YT529. Diagnostic value of **recombinant allergens**.. Pauli G (Reprint). Hop Univ Strasbourg, Serv Pneumol, BP 426, F-67091 Strasbourg, France (Reprint); Hop Univ Strasbourg, Serv Pneumol, F-67091 Strasbourg, France. REVUE

FRANCAISE D ALLERGOLOGIE ET D IMMUNOLOGIE CLINIQUE (1997) Vol. 37, No. 8, pp. 1093-1101. ISSN: 0335-7457. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE. Language: French.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Genetic engineering techniques applied to allergens have allowed the production of **recombinant allergens**. Validation of **recombinant allergens** demands confirmation of their immunological activity and their identity with natural allergens by in vivo and in vitro techniques on a sufficiently large number of allergic subjects. The results currently available for the main respiratory allergens ar reported. For example, the validity of the birch **recombinant allergen** Bet v 1 was confirmed by in vitro tests, but also by skin tests and nasal and bronchial challenge tests. The combination of four **recombinant allergens** of timothy allowed the in vitro detection of sensitization to Graminaceae pollens in 94.5% of patients. The validity of up 2 **recombinant allergens** has been confirmed for house dust mites. Systems of expression allowing glycosylation of recombinant proteins were necessary to validate group 1 **recombinant allergen** proteins. **Recombinant allergen** Blo t 5 has been tested in vitro and in vivo, and was found to be effective in the detection of sensitization to Blomia tropicalis, a domestic allergen in subtropical countries. Only **recombinant allergen** Bla g 4 has been tested in vitro and in vivo, with positive reactions in almost 50% of subjects sensitized to crockroaches. Recombinant Asp f 1 was tested in subjects suffering from allergic bronchopulmonary aspergillosis, and was positive in 60 to 85% of cases. Studies are also available for **recombinant allergens** of phospholipase A2, the major allergen of bee venom. The consequences of the development of **recombinant allergens** are then analysed: better standardized allergens for diagnostic tests, study of the spectrum of specificities of the IgE induced by an allergen, quantification of specific IgE, better approach to cross-allergies using **recombinant allergens** of the main cross allergens. The application of **recombinant allergens** to basic research has led to production of **modified recombinant allergens** : synthetis of recombinant polypeptides corresponding to T epitopes, production of **recombinant allergens** isoforms with reduced allergenic activity, production of **recombinant allergens** of allergenic molecules **modified** by directed mutations. The use of these **modified recombinant allergens** is one line of research which, in the future, may lead to new modalities of specific desensitization. Other lines of research are also under investigation: inhibition of antigen-antibody reactions by the use of recombinant Fab-blocking molecules, and recombinant molecules: of immunodominant haptens.

L8 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 3

1993:90720 Document No.: PREV199395045916. Expression and thrombin cleavage of Poa p IX **recombinant allergens** fused to glutathione-S-transferase. Olsen, Egil; Mohapatra, Shyam S. [Reprint author]. Dep. Immunology, Univ. Manitoba, 608-730 Williams Avenue, Winnipeg, Man. R3E OW3, Canada. International Archives of Allergy and Immunology, (1992) Vol. 98, No. 4, pp. 343-348. CODEN: IAAIEG. ISSN: 1018-2438. Language: English.

AB The high-level expression and purification of Poa p IX recombinant grass pollen allergens were examined utilizing a **modified** pGEX plasmid, designated as pGEX 2T-1. This vector permits frame-1 ligation of lambda-gt11 cDNA inserts and cleavage of the recombinant allergenic protein from the fusion partner glutathione S-transferase. The expression

of the fusion proteins in water-soluble form varied among the transformants of the same bacterial strain and also between different host strains. Purification of the fusion proteins by affinity chromatography employing glutathione agarose gel revealed that proteases in the bacterial lysate bound to the gel and were co-eluted with the fusion proteins. These proteases, which specifically degraded the recombinant proteins to varying degrees, were inhibited by both of the inhibitors, phenylmethylsulfonyl fluoride and aprotinin. Cleavage by thrombin of the fusion proteins indicated that the structure of the individual protein affected the thrombin accessibility to the cleavage site. Increased concentration of thrombin partly compensated this effect, but resulted in a broader specificity of the enzyme. By contrast, cleavage of the fusion protein when it was still attached to the glutathione gel was convenient and led to purification of the product devoid of proteolytic activity. Since almost all the **recombinant allergens** have been cloned in lambda-gt11 vector, the pGEX 2T-1 vector reported herein will facilitate the synthesis, purification of the corresponding allergenic proteins or their peptides in soluble and biologically active forms.

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L2             0 S L1 AND HEAT-KILLED
L3             0 S L1 AND MODIFIED ALLERGEN
L4       1162694 S ESCHERICHIA COLI
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L6            19 S L5 AND MODIFIED
L7             0 S L6 AND HEAT-KILLED
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L9       387782 E COLI

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L10            0 L9 AND MODIFIED ALLERGEN

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L11         3911 L9 AND KILLED

=> s l11 and allergen
L12            1 L11 AND ALLERGEN

=> d l12 cbib abs

L12  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
2004:1121739   Document No. 142:278913  Sensitization and allergic response and
     intervention therapy in animal models.  Helm, Ricki M.; Burks, A. Wesley
     (Department of Microbiology/Immunology, University of Arkansas for Medical
     Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA).  Journal of AOAC
     International, 87(6), 1441-1447 (English) 2004.  CODEN: JAINEE.  ISSN:
     1060-3271.  Publisher: AOAC International.
AB   A review is presented of 3 murine models and a swine neonatal model used
     to investigate immunotherapeutic options.  In Model 1, mutation of linear
     IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1
     is discussed with respect to expression in transgenic tobacco plants and
     correct folding following expression in the pET16b construct.  In Model 2,
     the mutations of Ara h 1 were assessed for use as an immunotherapeutic
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agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with heat-**killed E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

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=> s heat-killed E coli
L13        230 HEAT-KILLED E COLI

=> s l13 and vaccine
L14         15 L13 AND VACCINE

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L15           9 DUP REMOVE L14 (6 DUPLICATES REMOVED)

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L15  ANSWER 1 OF 9     MEDLINE on STN
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2004340100. PubMed ID: 15189567. NF-kappaB p50 facilitates neutrophil accumulation during LPS-induced pulmonary inflammation. Mizgerd Joseph P; Lupa Michal M; Spieker Matt S. (Physiology Program, Harvard School of Public Health, Boston, MA, 02115 USA.. jmizgerd@hsph.harvard.edu) . BMC immunology [electronic resource], (2004 Jun 9) Vol. 5, pp. 10. Electronic Publication: 2004-06-09. Journal code: 100966980. E-ISSN: 1471-2172. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Transcription factors have distinct functions in regulating immune responses. During Escherichia coli pneumonia, deficiency of NF-kappaB p50 increases gene expression and neutrophil recruitment, suggesting that p50 normally limits these innate immune responses. p50-deficient mice were used to determine how p50 regulates responses to a simpler, non-viable bacterial stimulus in the lungs, E. coli lipopolysaccharide (LPS). RESULTS: In contrast to previous results with living E. coli, neutrophil accumulation elicited by E. coli LPS in the lungs was decreased by p50 deficiency, to approximately 30% of wild type levels. **Heat-killed E. coli** induced neutrophil accumulation which was not decreased by p50 deficiency, demonstrating that bacterial growth and metabolism were not responsible for the different responses to bacteria and LPS. p50 deficiency increased the LPS-induced expression of kappaB-regulated genes essential to neutrophil recruitment, including KC, MIP-2, ICAM-1, and TNF-alpha suggesting that p50 normally limited this gene expression and that decreased neutrophil recruitment did not result from insufficient expression of these genes. Neutrophils were responsive to the chemokine KC in the peripheral blood of p50-deficient mice with or without LPS-induced pulmonary inflammation. Interleukin-6 (IL-6), previously demonstrated to decrease LPS-induced neutrophil recruitment in the lungs, was increased by p50 deficiency, but LPS-induced neutrophil recruitment was decreased by p50 deficiency even in IL-6 deficient mice. CONCLUSION: p50 makes essential contributions to neutrophil accumulation elicited by LPS in the lungs. This p50-dependent pathway for neutrophil accumulation can be overcome by bacterial products other than LPS and does not require

IL-6.

L15 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1
96101747. PubMed ID: 7494229. Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing Escherichia coli. Fenton R G; Keller C J; Hanna N; Taub D D. (Division of Clinical Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.) Journal of the National Cancer Institute, (1995 Dec 20) Vol. 87, No. 24, pp. 1853-61. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Point mutations in the ras proto-oncogene that activate its oncogenic potential occur in approximately 30% of human cancers. Previous studies have demonstrated that T-cell immunity against some forms of mutant Ras proteins could be elicited, and some effectiveness against tumors expressing activated Ras has been reported. PURPOSE: The goal of this study was to determine if immunization of mice with two forms of mutant Ras protein can induce high levels of Ras mutation-specific T-cell immunity in vitro and tumor regression in vivo. METHODS: Mice (BALB/c or C3H/HeJ) were immunized subcutaneously at 2-week intervals with purified Ras oncoproteins mixed with the immunologic adjuvants Antigen Formulation or QS-21, both of which have been shown to enhance the induction of T-cell-mediated immunity when included as components of soluble protein **vaccines**. In some experiments, mice were immunized directly with heat-killed Escherichia coli that had been induced to express one of the mutant Ras proteins. Spleen cells plus lymph node cells from Ras-immunized mice were tested in vitro for lysis of syngeneic Ras-expressing tumor cells and proliferation in response to mutant Ras peptides. For some of the cytolytic activity experiments, the spleen cells were grown under TH1 conditions (growth in presence of interleukin 2, interferon gamma, and an antibody directed against interleukin 4 to stimulate a cell-mediated immune response) or TH2 conditions (growth in presence of interleukins 2 and 4 to stimulate a humoral immune response). The specificity of immunity was examined in vivo by challenge of Ras-immunized mice with syngeneic tumor cells expressing mutant Ras oncoproteins (HaBalb, i.e., BALB/c mouse cells expressing Ras with arginine substituted at amino acid position 12 [Arg 12 Ras]; C3HL61, i.e., C3H/HeJ mouse cells expressing Ras with leucine substituted at position 61 [Leu 61 Ras]). Ten mice per group were used in each experiment. RESULTS: Proliferative and cytolytic T-cell responses directed against the Arg 12 Ras protein were generated in BALB/c mice, resulting in protection against challenge with cells expressing Arg 12 Ras and therapeutic benefit in mice bearing established tumors expressing this protein. In C3H/HeJ mice, high levels of cytolytic and proliferative responses were induced against Leu 61 Ras. Immunization with **heat-killed E. coli** genetically engineered to express Leu 61 Ras also led to the induction of anti-Ras T-cell immunity. T cells grown under TH1 conditions were cytolytic against Ras-transformed tumor cells, whereas those grown under TH2 conditions were not. CONCLUSIONS: Immunization as described here leads to Ras mutation-specific antitumor immunity in vitro and in vivo, with therapeutic efficacy in an established tumor model.

L15 ANSWER 3 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
1985:233270 Document No.: PREV198579013266; BA79:13266. IMMUNOCONGLUTININ LEVELS IN CHICKS VACCINATED WITH SALMONELLA-GALLINARUM 9R SALMONELLA-PULLORUM E-79 OR ESCHERICHIA-COLI 020 **VACCINES** AND EXPERIMENTALLY INFECTED WITH SALMONELLA-GALLINARUM. JAISWAL T N [Reprint author]; MITTAL K R. COLLEGE OF VET SCIENCE AND ANIMAL HUSBANDRY, GUJARAT AGRIC UNIVERSITY, SK NAGAR, DANTIWADA, BANASKANTHA-395 506. Indian Veterinary Medical Journal, (1984) Vol. 8, No. 1, pp. 9-13. CODEN: IVMJDL. ISSN: 0250-5266. Language: ENGLISH.

AB Vaccination of chicks with live S. gallinarum (9R) **vaccines** with

or without adjuvant caused an initial fall in the levels of pre-existing autostimulated immunoconglutinin (IK) by the 10th day but a slow increase in the IK level by 21st day postvaccination. Heat-killed S. pollorum (E79) and **heat-killed E. coli** (020) **vaccines** caused no such reduction in the IK level during the post-vaccination period. An increase in the IK level during post-vaccination period in these groups of chickens were observed. Challenge infection with S. gallinarium (V) in all the vaccinated groups of birds showed a marked decrease in IK level during the early challenge period indicating the involvement of IK in the host parasite reaction. The IK level increased by the 21st day post-challenge. Evidently, involvement of IK may help in host defense only in initial stages but eventually fail to protect chicks against S. gallinarum infection when the causative agent manages to enter the cells when both specific antibodies and nonspecific serum factor like IK fail to be effective.

L15 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 2

82190380. PubMed ID: 7042755. Induction of immunity against lethal Haemophilus influenzae type b infection by Escherichia coli core lipopolysaccharide. Marks M I; Ziegler E J; Douglas H; Corbeil L B; Braude A I. The Journal of clinical investigation, (1982 Apr) Vol. 69, No. 4, pp. 742-9. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Efforts to prevent Haemophilus influenzae type b (HIB) infections in infancy have been hampered by the low immunogenicity of capsular polysaccharide **vaccines** in children younger than 18 mos. In searching for alternate immunogens, we have studied the protective potential of polysaccharide-poor, lipid-rich endotoxin (LPS) core in experimental HIB infections. Because all gram-negative bacteria have similar LPS core structures, we were able to use as **vaccine** the J5 mutant of Escherichia coli 0111, the LPS of which consists only of core components, and thus to avoid problems in interpretation arising from **vaccine** contamination with non-LPS HIB immunogens. Mice were given graded inocula of HIB and developed lethal infection analogous to human HIB disease when virulence was enhanced with mucin and hemoglobin. After active immunization with **heat-killed E. coli** J5, 40/50 (80%) of infected mice survived, compared with 14/50 (28%) of saline-immunized controls (P less than 0.005). Passive immunization with rabbit antiserum against E. coli J5 prevented lethal HIB infection when administered 24 or 72 h before or 3 h after infection. This protection was abolished by adsorption of antiserum with purified J5 LPS, with survival reduced from 14/24 to 0/24 (P less than 0.005). Furthermore, rabbit antiserum to purified J5 LPS gave just as potent protection against death as antiserum to whole J5 cells. These studies demonstrate that immunity to core LPS confers protection against experimental murine HIB infection and provide the framework for a new approach to prevention of human disease from HIB.

L15 ANSWER 5 OF 9 MEDLINE on STN

81281536. PubMed ID: 7023456. Consequences of active or passive immunization of turkeys against Escherichia coli O78. Arp L H. Avian diseases, (1980 Oct-Dec) Vol. 24, No. 4, pp. 808-15. Journal code: 0370617. ISSN: 0005-2086. Pub. country: United States. Language: English.

AB Turkeys were injected at 7 and 14 days of age with live, heat-killed or formalin-killed Escherichia coli O78. Other turkeys were passively immunized at 22 days of age with hyperimmune serum produced against live or **heat-killed E. coli** O78. All turkeys were challenged at 24 days of age with E. coli O78. Turkeys immunized intramuscularly or intratracheally with live E. coli O78 were protected from death, whereas few turkeys given killed E. coli O78 were protected. Passively immunized turkeys were protected from death regardless of whether live or **heat-killed E.**

coli O78 was used to produce the hyperimmune serum. Most turkeys that survived challenge developed septic polysynovitis 2--4 days after challenge.

L15 ANSWER 6 OF 9 MEDLINE on STN
76189304. PubMed ID: 818014. Antiviral activity of Brucella abortus preparations; separation of active components. Feingold D S; Keleti G; Youngner J S. Infection and immunity, (1976 Mar) Vol. 13, No. 3, pp. 763-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Injection into mice of heat-killed Brucella abortus or aqueous ether-extracted B. abortus (Bru-pel) induced a "virus-type" interferon response, with peak titers at 6.5 h. The animals also were protected against challenge with otherwise lethal doses of Semliki forest virus. Extraction of either heated B. abortus or BRU-PEL with a mixture of chloroform-methanol (2:1, vol/vol) (C-M(yielded an insoluble residue (extracted cells) and a C-M extract. Neither extracted cells nor C-M extract alone induced interferon or afforded protection against Semliki forest virus infection in mice. Full interferon-inducing and protective activity was restored when extracted cells were recombined with C-M extract. C-M extract from heat-killed Escherichia coli also was effective in restoring activity to extracted Brucella cells. Neither **heat -killed E. coli** nor its C-M extract was active, nor was C-M estracted E. coli recombined with the C-M extract from B. abortus. These results suggest that the interferon-inducing and antiviral protective properties of B. abortus are constituted of a C-M-extractable component that is common to B. abortus and E. coli and an unextractable component that is unique to B. abortus.

L15 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
1974:567727 Document No. 81:167727 Intestinal antibody secretion in the young pig in response to oral immunization with Escherichia coli. Porter, P.; Kenworthy, R.; Noakes, D. E.; Allen, W. D. (Unilever Res., Sharnbrook/Bedford, UK). Immunology, 27(5), 841-53 (English) 1974. CODEN: IMMUAM. ISSN: 0019-2805.
AB Intestinal immunoglobulins and antibodies in the local immune response to E. coli O somatic antigens was studied in young fistulated pigs. Antibody levels in intestinal secretion were raised for .apprx.2-3 weeks following a single local antigenic challenge with a heat-killed aqueous suspension of E. coli. A 2nd challenge provoked a similar response suggesting a lack of immunol. memory. Antibody activity in the secretions was predominantly associated with IgA and immunofluorescent studies of biopsy specimens from these pigs indicated that intestinal synthesis and secretion of IgA had begun by the 10th day of life. Studies of piglets reared with the sow indicated that oral immunization with E. coli antigen after 10 days of age stimulated intestinal antibody secretion before weaning at 3 weeks. The response of gnotobiotic pigs to oral immunization and infection was evaluated by immunofluorescent histol. of the intestinal mucosa. Repeated oral administration of **heat-killed E. coli** O8 gave an immunocyte response in the lamina propria numerically comparable with that produced by infection. The early response was dominated by cells of the IgM class whereas after 3 weeks IgA cells predominated. In the germ-free animal very few immunoglobulin-containing cells were detected. In vitro studies of antibacterial activity indicated that the most probable mechanism of immunol. control in the alimentary tract is bacteriostasis.

L15 ANSWER 8 OF 9 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
75027648 EMBASE Document No.: 1975027648. The effect of active immunisation on ascending pyelonephritis in the rat. Radford N.J.; Chick S.; Ling R.; et al.. KRUF Inst. Ren. Dis., Welsh Nat. Sch. Med., Roy. Infirm., Cardiff,

United Kingdom. J.PATH. Vol. 112, No. 3, pp. 169-175 1974.
CODEN: JPBAA7
Language: English.

AB In the rat, active immunization with **heat killed E. coli** serotype 078 **vaccine** produced a high titer of IgM anti O antibody after 14 days. At this time, lower titers of IgG anti O antibodies were found in some of the animals. These antibodies did not prevent bacterial invasion of the kidney nor did they affect the incidence or severity of the renal scarring following ascending infection with E. coli serotype 078. Fourteen days after immunization with a formalin killed **vaccine** very high titers of IgM and IgG anti K antibodies were noted; these were in excess of 1 in 5120. It was shown that these antibodies reduced the severity but not the frequency of renal scarring following ascending E. coli infection.

L15 ANSWER 9 OF 9 MEDLINE on STN

71078403. PubMed ID: 4923787. [Oral immunization against coli enteritis with streptomycin-dependent E. coli. V. Different efficiency of live Sm-d and **heat killed E. coli** O111 B4 **vaccine** in settling of the homologous Sm-r strain in mice with antibiotic sterilized intestine]. Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Coli-Keimen. V. Unterschiedliche Wirksamkeit von Impfstoffen aus lebenden Streptomycin-dependenten und hitzeabgetoteten EC-O111 B4-Bakterien auf die Hemmung der Ansiedlung des homologen Streptomycin-resistenten Stammes bei darmsterilen Mausen. Lindek; Koch H. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale, (1970) Vol. 215, No. 3, pp. 286-95. Journal code: 0337744. ISSN: 0372-8110. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

=> s l11 and allergen
L16 1 L11 AND ALLERGEN

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L16 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN

2004:1121739 Document No. 142:278913 Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M.; Burks, A. Wesley (Department of Microbiology/Immunology, University of Arkansas for Medical Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.

AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with heat-**killed E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl.

study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

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L17 0 L11 AND PET16

=> s recombinant modified allergen
L18 2 RECOMBINANT MODIFIED ALLERGEN

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L19 ANSWER 1 OF 1 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 1
2004135126 EMBASE [Hymenoptera venom allergy: Recent developments and perspectives in diagnosis and immunotherapy]. ALLERGIES AUX VENINS D'HYMENOPTERES: PERSPECTIVES DE PROGRES DANS LE DIAGNOSTIC ET LE TRAITEMENT D'HYPOSENSIBILISATION. Muller U.R.. U.R. Muller, Medizinische Klinik, Spital Bern Ziegler, Morillonstrasse 75-91, Bern CH-3006, Bern, Switzerland. ulrich.mueller@spitalbern.ch. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 44, No. 3, pp. 281-285 2004.
Refs: 34.
ISSN: 0335-7457. CODEN: RFAIBB
S 0335-7457(04)00017-6. Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20040412. Last Updated on STN: 20040412
AB There is a considerable potential to improve both diagnosis and immunotherapy in patients allergic to hymenoptera venoms. Among available diagnostic procedures, the basophil activation test appears interesting owing to its high specificity and sensitivity. This test is, however, expensive and without predictive value with regard to the protection induced by immunotherapy. Estimation of IL10 in lymphocyte cultures stimulated with the allergen may be more informative in this situation. An elevated basal serum tryptase level is a risk factor for particularly severe anaphylactic reactions and hence an indication for prolonged immunotherapy. Diagnostic tests with a cocktail of the major recombinant venom allergens have superior specificity compared to tests with the whole venom. **Recombinant modified allergens** or T cell epitope peptides no longer react with B cell epitopes of specific IgE while their reactivity with T cell epitopes is conserved. They will induce fewer side effects but they still be effective for immunotherapy. A reduction of side effects during the initial phase of immunotherapy can also be achieved by pre-medication with antihistamines. .COPYRGT. 2003 Elsevier SAS. Tous droits reserves.

=> s modified allergen
L20 557 MODIFIED ALLERGEN

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L21 0 L20 AND PEAUNT

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L22 21 L20 AND PEANUT

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L23 ANSWER 1 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
2006:1035827 The Genuine Article (R) Number: 094PN. Immunological mechanisms of allergen-specific immunotherapy. Larche M (Reprint); Akdis C A; Valenta R. McMaster Univ, Dept Med, Div Clin Immunol & Allergy, 1200 Main St W, Hamilton, ON L8N 3Z5, Canada (Reprint); McMaster Univ, Dept Med, Div Clin Immunol & Allergy, Hamilton, ON L8N 3Z5, Canada; Univ London Imperial Coll Sci Technol & Med, MRC & Asthma UK Ctr Allerg Mechanisms Asthma, Dept Allergy & Clin Immunol, Natl Heart & Lung Inst, Fac Med, London SW7 2AZ, England; Swiss Inst Allergy & Asthma Res, CH-7270 Davos, Switzerland; Med Univ Vienna, Div Immunopathol, Dept Pathophysiol, Ctr Physiol & Pathophysiol, A-1090 Vienna, Austria. m.larche@imperial.ac.uk. NATURE REVIEWS IMMUNOLOGY (OCT 2006) Vol. 6, No. 10, pp. 761-771. ISSN: 1474-1733 . Publisher: NATURE PUBLISHING GROUP, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Allergen-specific immunotherapy has been carried out for almost a century and remains one of the few antigen-specific treatments for inflammatory diseases. The mechanisms by which allergen-specific immunotherapy exerts its effects include the modulation of both T-cell and B-cell responses to allergen. There is a strong rationale for improving the efficacy of allergen-specific immunotherapy by reducing the incidence and severity of adverse reactions mediated by IgE. Approaches to address this problem include the use of **modified allergens**, novel adjuvants and alternative routes of administration. This article reviews the development of allergen-specific immunotherapy, our current understanding of its mechanisms of action and its future prospects.

L23 ANSWER 2 OF 11 MEDLINE on STN DUPLICATE 1
2005530273. PubMed ID: 16189800. Allergenic characteristics of a modified **peanut** allergen. King Nina; Helm Ricki; Stanley J Steven; Vieths Stefan; Luttkopf Dirk; Hatahet Lina; Sampson Hugh; Pons Laurent; Burks Wesley; Bannon Gary A. (Department of Biochemistry & Molecular Biology, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA.) Molecular nutrition & food research, (2005 Oct) Vol. 49, No. 10, pp. 963-71. Journal code: 101231818. ISSN: 1613-4125. Pub. country: Germany: Germany, Federal Republic of. Language: English.
AB Attempts to treat **peanut** allergy using traditional methods of allergen desensitization are accompanied by a high risk of anaphylaxis. The aim of this study was to determine if modifications to the IgE-binding epitopes of a major **peanut** allergen would result in a safer immunotherapeutic agent for the treatment of **peanut**-allergic patients. IgE-binding epitopes on the Ara h 2 allergen were modified, and modified Ara h 2 (mAra h 2) protein was produced. Wild-type (wAra h 2) and mAra h 2 proteins were analyzed for their ability to interact with T-cells, their ability to bind IgE, and their ability to release mediators from a passively sensitized RBL-2H3 cell line. Multiple T-cell epitopes were identified on the major **peanut** allergen, Ara h 2. Ara h 2 amino acid regions 11-35, 86-125, and 121-155 contained the majority of peptides that interact with T-cells from most patients. The wAra h 2 and mAra h 2 proteins stimulated proliferation of T-cells from **peanut** -allergic patients to similar levels. In contrast, the mAra h 2 protein exhibited greatly reduced IgE-binding capacity compared to the wild-type allergen. In addition, the **modified allergen** released

significantly lower amounts of beta-hexosaminidase, a marker for IgE-mediated RBL-2H3 degranulation, compared to the wild-type allergen.

L23 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.
AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use **peanut** allergens to illustrate applications of the invention.

L23 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 2
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318.

PRIORITY: US 2001-276822P 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use **peanut** allergens to illustrate applications of the invention.

L23 ANSWER 5 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2003:28190 The Genuine Article (R) Number: 625KX. Clinical aspects of food allergy. Papageorgiou P S (Reprint). 58 Voutsina St, Holargos 15561, Greece (Reprint); Univ Athens, Sch Med, P&A Kyriakou Childrens Hosp, Allergy Unit, GR-11527 Athens, Greece. BIOCHEMICAL SOCIETY TRANSACTIONS (NOV 2002) Vol. 30, Part 6, pp. 901-906. ISSN: 0300-5127. Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Food allergy affects 2.5 % of adults and 6-8 % of children, and is a leading cause of life-threatening anaphylactic episodes. Food allergy is defined as an adverse reaction to foods that is mediated immunologically and involves specific IgE or non-IgE mechanisms. In this review only IgE-related food allergy will be considered. Many food allergens are glycoproteins, but they do not share any striking biochemical similarities. The definition of many food proteins at the molecular level has tremendously facilitated our understanding of clinical syndromes and seemingly bizarre observations. Clinical manifestations of food allergy include symptoms of the gastrointestinal, cutaneous and respiratory systems, as well as systemic anaphylaxis. The diagnosis of food allergy involves a stepwise approach, including medical history taking, demonstration of specific IgE and confirmation by oral food challenge. The management of the food-allergic patient at present consists of avoidance of the culprit food and education, while future advances may include specific immunotherapy with **modified allergens** or DNA vaccination.

L23 ANSWER 6 OF 11 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 3

2002:301398 Document No.: PREV200200301398. Immunotherapy for **peanut** allergy using **modified allergens** and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.

Meeting Info.: 58th Annual Meeting of the American Academy of Allergy,

Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L23 ANSWER 7 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
2002:530650 The Genuine Article (R) Number: 563MD. Modification of **peanut** allergen Ara h 3: Effects on IgE binding and T cell stimulation. Rabjohn P; West C M; Connaughton C; Sampson H A; Helm R M (Reprint); Burks A W; Bannon G A. Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Slot 512, 1120 Marshall St, Little Rock, AR 72202 USA (Reprint); Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Little Rock, AR 72202 USA; Univ Arkansas Med Sci, ACHRI, Dept Pediat, Little Rock, AR 72202 USA; Mt Sinai Sch Med, Dept Pediat, New York, NY USA. INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (MAY 2002) Vol. 128, No. 1, pp. 15-23. ISSN: 1018-2438. Publisher: KARGER, ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Background: **Peanut** allergy is a major health concern due to the increased prevalence, potential severity, and chronicity of the reaction. The cDNA encoding a third **peanut** allergen, Ara h 3, has been previously cloned and characterized. Mutational analysis of the Ara h 3 IgE-binding epitopes with synthetic peptides revealed that single amino acid changes at critical residues could diminish IgE binding. Methods: Specific oligonucleotides were used in polymerase chain reactions to modify the cDNA encoding Ara h 3 at critical IgE binding sites. Four point mutations were introduced into the Ara h 3 cDNA at codons encoding critical amino acids in epitopes 1, 2, 3 and 4. Recombinant modified proteins were used in SDS-PAGE/Western IgE immunoblot, SDS-PAGE/Western IgE immunoblot inhibition and T cell proliferation assays to determine the effects of these changes on in vitro clinical indicators of **peanut** hypersensitivity. Results: Higher amounts of modified Ara h 3 were required to compete with the wild-type allergen for **peanut** -specific serum IgE. Immunoblot analysis with individual serum IgE from Ara-h-3-allergic patients showed that IgE binding to the modified protein decreased similar to35-85% in comparison to IgE binding to wildtype Ara h 3. Also, the modified Ara h 3 retained the ability to stimulate T cell activation in PBMCs donated by Ara-h-3-allergic patients. Conclusions: The engineered hypoallergenic Ara h 3 variant displays two characteristics essential for recombinant allergen immunotherapy; it has a reduced binding capacity for serum IgE from **peanut**-hypersensitive patients and it can stimulate T-cell proliferation and activation. Copyright (C) 2002 S, Karger AG, Basel.

L23 ANSWER 8 OF 11 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 4
2001120857 EMBASE [**Peanut** allergy]. ALLERGIE A L'ARACHIDE. Dutau G.; Rance F.. G. Dutau, Unite des maladies respiratoires, Hopital des Enfants, 330, avenue de Grande-Bretagne, 31026 Toulouse Cedex 3, France. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 41, No. 2, pp. 187-198 2001.
Refs: 98.
ISSN: 0335-7457. CODEN: RFAIBB
Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20010412. Last Updated on STN: 20010412
AB **Peanut** allergy, which is frequent in the United States and was much less so in Europe up to the mid-eighties, has become a major problem in many industrialized countries. **Peanut** consumption is high in Eastern Europe, the United Kingdom, The Netherlands, Germany and France. The frequency of **peanut** allergy is between 0.5 and O.7% in the general population. Two million Americans are now thought to be affected. In France **peanuts** are one of the most frequent allergens, lying

second (27.4 %) to egg in food allergies in children, and holding first place in food allergies in children aged over 3 years. Sensitization occurs through ingestion, contact even if indirect, and inhalation. The symptoms, which affect the skin and the respiratory or gastrointestinal tract, appear a few minutes to a few hours after exposure. Serious reactions (anaphylactic shock, life-threatening reactions, sudden death) have been described. Asthma has a significantly higher association with **peanut** allergy than with other allergies, taken overall. As with other food allergies, diagnosis is based on history, prick-tests, screening for specific serum IgE and food challenge whose modalities (labial and oral challenge) are debated. For the time being, elimination is the only form of treatment. The development of a **modified allergen** as immunogenic as possible but practically without allergenic effects should give immunotherapy new impetus. Patients with severe **peanut** allergy should carry a card or wear a distinctive bracelet indicating their condition as well as an emergency kit including in particular epinephrine. .COPYRGT. 2001 Editions scientifiques et medicales Elsevier SAS.

L23 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 5

2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major **peanut** allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) Vol. 124, No. 1-3, pp. 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of **peanut** allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from **peanut** anaphylaxis, there is still no safe, effective, specific therapy for the **peanut**-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major **peanut** allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the **modified allergen**, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating **peanut**-sensitive patients. Modified **peanut** allergens were subjected to immunoblot analysis using **peanut**-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of **peanut** anaphylaxis. RESULTS: In general, the **modified allergens** were poor competitors for binding of **peanut**-specific IgE when compared to their wild-type counterpart. The **modified allergens** demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the **modified allergens** retained the ability to stimulate T cell proliferation. CONCLUSIONS: These **modified allergen** genes and proteins should provide a safe immunotherapeutic agent for the treatment of **peanut** allergy.

L23 ANSWER 10 OF 11 CAPLUS

1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compound that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use **peanut** allergens to demonstrate alteration of IgE binding sites. The critical amino acids within each of the IgE binding epitopes of the **peanut** protein that are important to Ig binding have been determined Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most critical to IgE binding.

L23 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN

1983:556476 Document No. 99:156476 Some immunochemical studies of native and **modified allergens**. King, Te Piao; Giallongo, Agata (Rockefeller Univ., New York, NY, 10021, USA). Skandia International Symposia, Volume Date 1982, 15th(Theor. Clin. Aspects Allerg. Dis.), 215-36 (English) 1983. CODEN: SISYDD. ISSN: 0346-9069.

AB Studies were performed to induce significant suppression of specific IgE with very low doses of a highly immunogenic material (modified antigens). Two allergen-lectin conjugates were prepared; ragweed antigen E was conjugated with either **peanut** agglutinin or wheat germ agglutinin. These **modified allergens** were not more effective than the native allergen in suppressing specific IgE production

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L26 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN

2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.;

Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L26 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

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L26 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.
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L28 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
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L28 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

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L28 ANSWER 3 OF 6 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 2

2001120857 EMBASE [Peanut allergy]. ALLERGIE A L'ARACHIDE. Dutau G.; Rance F.. G. Dutau, Unite des maladies respiratoires, Hopital des Enfants, 330, avenue de Grande-Bretagne, 31026 Toulouse Cedex 3, France. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 41, No. 2, pp. 187-198 2001.
Refs: 98.
ISSN: 0335-7457. CODEN: RFAIBB
Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20010412. Last Updated on STN: 20010412

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L28 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN

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APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

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L28 ANSWER 5 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:146495 The Genuine Article (R) Number: YY214. Tolerogenic activity of polyethylene glycol-conjugated lysozyme distinct from that of the native counterpart. Ito H O (Reprint); So T; Hirata M; Koga T; Ueda T; Imoto T. Kyushu Univ, Fac Dent, Dept Biochem, Fukuoka 81282, Japan (Reprint); Kyushu Univ, Grad Sch Pharmaceut Sci, Fukuoka 81282, Japan. IMMUNOLOGY (FEB 1998) Vol. 93, No. 2, pp. 200-207. ISSN: 0019-2805. Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Conjugation of proteins with polyethylene glycol (PEG) has been reported to make the proteins tolerogenic. Native proteins are also potentially tolerogenic when given without adjuvants. We compared immunotolerogenic activities between PEG-conjugated and native hen **egg**-white lysozyme (HEL). BALB/c mice were given consecutive weekly intraperitoneal administrations of PEG-conjugated HEL, unmodified HEL or phosphate-buffered saline (PBS), for 3 weeks, then challenged with HEL in Freund's complete adjuvant. The pretreatment with PEG-HEL tolerized both T-cell and humoral responses, while native HEL tolerized only the T-cell response. Immunoglobulin G1 (IgG1) antibody was already elevated in HEL-pretreated mice prior to challenge immunization, followed by suppressed IgG2a and IgG2b. but spared IgG1 production after the antigen challenge. whereas PEG-HEL-pretreated mice produced no antibody in all IgG subclasses prior and subsequent to the antigen-challenge. Production of interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) by lymphoid cells in response to HEL in vitro was markedly suppressed in both the antigen-pretreated groups. while suppression of IL-4 production was evident only in PEG-HEL-, not in HEL-pretreated animals. Involvement of suppressor cells in these tolerance states was found to be unlikely. and the immunological property of PEG-HEL differed from deaggregated HEL that was similar to the original HEL. These results suggest a unique immunotolerogenic activity of PEG-conjugated proteins to suppl css both T-helper type-1 (Th1)and Th2-type responses, the result bring extensive inhibition of all IgG subclass responses, while tolerance induction by unconjugated soluble proteins tends to be targeted on Th1-, but spares Th2-type responses.

L28 ANSWER 6 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1996:258810 The Genuine Article (R) Number: UC578. Reduced immunogenicity of monomethoxypolyethylene glycol-modified lysozyme for activation of T cells . So T (Reprint); Ito H O; Koga T; Ueda T; Imoto T. KYUSHU UNIV, GRAD SCH PHARMACEUT SCI, FUKUOKA 81282, JAPAN; KYUSHU UNIV, SCH DENT, DEPT BIOCHEM, FUKUOKA 81282, JAPAN. IMMUNOLOGY LETTERS (JAN 1996) Vol. 49, No. 1-2, pp. 91-97. ISSN: 0165-2478. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chemical modification of proteins with monomethoxypolyethylene glycol (mPEG) will reduce the immunogenicity of proteins. In the present study, we evaluated the effect of mPEG modification on the capacity of hen **egg**-white lysozyme (HEL) to stimulate T cells. Lymph node cells (LNCs) from mice immunized with HEL or with mPEG-HEL conjugate were cultured with these antigens, then we measured the proliferation and IL-2 production. mPEG-modification lowered the T cell-activating capacity of HEL, both in vitro and in vivo. Neither toxicity, nor antigen non-specific immunosuppressive capacity was observed with mPEG-HEL and unconjugated mPEG. Suppressor cells were unlikely to be generated in the mPEG-HEL-primed LNCs. We next examined the behavior of mPEG-HEL during antigen processing. The capacity of HEL and mPEG-HEL to be incorporated by live cells was much the same. However, the susceptibility to various proteases, including endosomal/lysosomal enzymes, was significantly decreased by mPEG modification. The increased resistance of mPEG-HEL to proteolytic degradation implied that the conjugate was poorly presented to T cells, This may be an important factor related to the low immunogenicity of mPEG modified proteins.

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L29          5 L20 AND SHRIMP

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L30           1 DUP REMOVE L29 (4 DUPLICATES REMOVED)

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L30 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
2000290936. PubMed ID: 10828721. Modulation of allergen-specific immune responses to the major **shrimp** allergen, tropomyosin, by specific targeting to scavenger receptors on macrophages. Rajagopal D; Ganesh K A; Subba Rao P V. (Department of Biochemistry, Indian Institute of Science, Bangalore, India.) International archives of allergy and immunology, (2000 Apr) Vol. 121, No. 4, pp. 308-16. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Tropomyosin from **shrimp** is the major cross-reacting crustacean food allergen. Earlier studies have led to the purification and immunochemical characterization of the major IgE binding epitopes of the allergen. Maleylated proteins are known to be specifically targeted to scavenger receptors on macrophage. Since antigens processed and presented by macrophages are known to elicit Th1 type of responses and allergic responses are characterized by polarization towards Th2 phenotype, the possibility of modulation of allergen-specific immune responses by targeting of tropomyosin to macrophage via scavenger receptor was explored. METHODS: The IgG and IgE binding potential of the native maleylated form of tropomyosin was carried out by ELISA and immunoblot. The ability of the native and maleylated form of allergen to induce in vitro proliferation of splenocytes from BALB/C mice immunized with both forms of allergen was tested. The in vitro production of IL-4 and IFN-gamma by splenocytes from mice immunized with the two forms of allergen was determined from culture supernatants. The in vivo production of serum IgG1 and IgG2a antibodies following immunization with native and

modified allergens was monitored by ELISA. RESULTS: The maleylated form of tropomyosin was found to have reduced antigenicity and allergenicity as compared to its native counterpart. The **modified allergen** was, however, found to elicit a cellular response similar to native tropomyosin in vitro. Analysis of the cytokine profiles showed a modulation from an IL-4-dominant, proallergic, Th2 phenotype to an IFN-gamma-dominant, antiallergic, Th1 phenotype that could also be correlated to a modulation in the in vivo antibody isotype. CONCLUSION: The results suggest the possible potential for modulating allergic responses in vivo by selective targeting to macrophages.


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     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 12:13:14 ON
     09 JAN 2007
L1          8476 S RECOMBINANT ESCHERICHIA COLI
L2             0 S L1 AND HEAT-KILLED
L3             0 S L1 AND MODIFIED ALLERGEN
L4       1162694 S ESCHERICHIA COLI
L5           462 S L4 AND RECOMBINANT ALLERGEN
L6            19 S L5 AND MODIFIED
L7             0 S L6 AND HEAT-KILLED
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L10            0 S L9 AND MODIFIED ALLERGEN
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L12            1 S L11 AND ALLERGEN
L13          230 S HEAT-KILLED E COLI
L14           15 S L13 AND VACCINE
L15            9 DUP REMOVE L14 (6 DUPLICATES REMOVED)
L16            1 S L11 AND ALLERGEN
L17            0 S L11 AND PET16
L18            2 S RECOMBINANT MODIFIED ALLERGEN
L19            1 DUP REMOVE L18 (1 DUPLICATE REMOVED)
L20          557 S MODIFIED ALLERGEN
L21            0 S L20 AND PEAUNT
L22           21 S L20 AND PEANUT
L23           11 DUP REMOVE L22 (10 DUPLICATES REMOVED)
L24            0 S L23 AND E COLI
L25            4 S L20 AND MILK
L26            3 DUP REMOVE L25 (1 DUPLICATE REMOVED)
L27            8 S L20 AND EGG
L28            6 DUP REMOVE L27 (2 DUPLICATES REMOVED)
L29            5 S L20 AND SHRIMP
L30            1 DUP REMOVE L29 (4 DUPLICATES REMOVED)

=> s l13 and inclusion body
L31            0 L13 AND INCLUSION BODY

=> s l13 and allergen
L32            1 L13 AND ALLERGEN

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L32  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
2004:1121739   Document No. 142:278913  Sensitization and allergic response and
     intervention therapy in animal models.  Helm, Ricki M.; Burks, A. Wesley
     (Department of Microbiology/Immunology, University of Arkansas for Medical
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Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.

AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with **heat-killed** **E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

=> s l11 and formaldehyde
L33 42 L11 AND FORMALDEHYDE

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L34 0 L33 AND ALLERGEN

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L35 19 DUP REMOVE L33 (23 DUPLICATES REMOVED)

=> d l35 1-19 cbib abs

L35 ANSWER 1 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

2006390023 EMBASE Detection of Escherichia coli O157:H7 using chicken immunoglobulin Y. Sunwoo H.H.; Wang W.W.; Sim J.S.. H.H. Sunwoo, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada. hsunwoo@ualberta.ca. Immunology Letters Vol. 106, No. 2, pp. 191-193 15 Aug 2006.
Refs: 8.
ISSN: 0165-2478. CODEN: IMLED6
S 0165-2478(06)00132-5. Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 20060831. Last Updated on STN: 20060831

AB A sandwich ELISA technique was examined to detect Escherichia coli O157:H7 using chicken anti-**E. coli** O157:H7 IgY as the capture-antibody and an anti-**E. coli** O157 mouse mAb conjugated with biotin as the detection antibody. The anti-**E. coli** O157:H7 IgY was harvested from eggs laid by hens (23 weeks of age, Single Comb White Leghorn) immunized with formalin-**killed** **E. coli** O157:H7. The IgY was purified by water dilution methods and gel chromatography on Sephacryl S-300 followed by ammonium sulfate precipitation. The sensitivity (CFU/ml) of sandwich ELISA for the **E. coli** O157:H7 was repeatedly examined with 10 replicates of each sample and a standard curve was plotted. The sandwich ELISA can detect as low as 40 CFU/ml of **E. coli** O157:H7. The data suggest that chicken IgY-based sandwich ELISA provides

a reliable, inexpensive and sensitive assay for the detection of the food-borne pathogen **E. coli** O157:H7.

L35 ANSWER 2 OF 19 EMBASE

2001304745 EMBASE Evaluation of in vitro antibacterial activity of some disinfectants on Escherichia coli serotypes. El-Naggar M.Y.M.; Akeila M.A.; Turk H.A.; El-Ebady A.A.; Sahaly M.Z.. Dr. M.Y.M. El-Naggar, Botany/Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt. Moustafa64@Yahoo.com. Journal of General and Applied Microbiology Vol. 47, No. 2, pp. 63-73 2001.
Refs: 33.
ISSN: 0022-1260. CODEN: JGAMA
Pub. Country: Japan. Language: English. Summary Language: English.
Entered STN: 20010913. Last Updated on STN: 20010913

AB Three disinfectants commonly used in poultry farms (formalin, TH4+, and Virkon-S) were chosen for the present study. The effect of disinfectant concentration and the duration of exposure to these disinfectants on the survival of Escherichia coli serotypes (O114:K-, O86, O55:K39, and O86:K60) were investigated. Formalin (0.6%), TH4+ (0.06%), and Virkon (0.5%) all **killed** the four serotypes within 5 min of exposure. As the disinfectant concentration decreases, the length of exposure time to kill serotype increases. At 0.03%, 0.007%, and 0.03% of formalin, TH4+ and Virkon-S concentrations failed to kill the four **E. coli** serotypes within 360 min, respectively. An improvement of the inhibitory effect of these disinfectants occurred when added together with the inoculum instead of an established population. The influence of formalin, TH4+, and Virkon-S on the cell morphology of **E. coli** O55:K39 was investigated by using transmission electron microscopy. Formalin-treated cells exhibited normal cell morphology, with the exception that the treated cell was less fimbriated, and more destruction of pili increased when formalin concentrations were doubled. Cells treated with TH4+ (0.03%) showed destruction of the cell wall and cell surface membrane after 5 min. Cell filamentation occurred at 0.015% and increased with the increase of exposure time to this drug. Spheroplasts were observed only when cells were treated with 0.125% Virkon-S for 60 min, and cell lysis started to occur when 0.25% Virkon-S was applied for 15 min. Scanning electron microscope study revealed that Virkon-S at 0.03% and TH4+ at 0.007% completely prevented the adherence of **E. coli** O55:K39 serotype to chicken tracheal organ, whereas formalin (0.03%) disinfection minimized the adherence of **E. coli** cells to tracheal explants after 360 min of incubation.

L35 ANSWER 3 OF 19 MEDLINE on STN DUPLICATE 1

2000137497. PubMed ID: 10675032. Identification and cloning of an aspartyl proteinase from Coccidioides immitis. Johnson S M; Kerekes K M; Zimmermann C R; Williams R H; Pappagianis D. (Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis 95616, USA.. smjohnson@ucdavis.edu) . Gene, (2000 Jan 11) Vol. 241, No. 2, pp. 213-22. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB A 45 kDa protein was isolated from a soluble vaccine prepared from **formaldehyde-killed** spherules of Coccidioides immitis. From the N-terminal amino acid sequence, the protein yielded a 17-amino-acid peptide that was homologous to sequences of other fungal aspartyl proteinases. The coccidioidal cDNA encoding the proteinase was amplified using oligonucleotide primers designed from the 45 kDa N-terminal amino acid sequence and a fungal aspartyl proteinase consensus amino acid sequence. The PCR product was cloned and sequenced, and the remaining 5' upstream and 3' downstream cDNA was amplified, cloned, and sequenced. The cDNA encoding the coccidioidal aspartyl proteinase open

reading frame was cloned and the fusion protein containing a C-terminal His-tag expressed in **E. coli**. The recombinant aspartyl proteinase was purified by immobilized metal affinity chromatography. This recombinant protein will be used for further studies to evaluate its antigenicity, including protective immunogenicity.

L35 ANSWER 4 OF 19 MEDLINE on STN DUPLICATE 2
1998311328. PubMed ID: 9648994. Escherichia coli and Proteus mirabilis inhibit the perinuclear but not the circulating antineutrophil cytoplasmic antibody reaction. Yang P; Danielsson D; Jarnerot G. (Dept. of Medicine, Orebro Medical Centre Hospital, Sweden.) Scandinavian journal of gastroenterology, (1998 May) Vol. 33, No. 5, pp. 529-34. Journal code: 0060105. ISSN: 0036-5521. Pub. country: Norway. Language: English.
AB BACKGROUND: Perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) are found in 48%-83% of serum samples from patients with ulcerative colitis (UC). Their pathogenic role and initiating stimuli are unknown. In contrast to patients with vasculitides and ANCA reactivities, the antibodies in UC patients do not react with myeloperoxidase (MPO) or proteinase 3 (PR3). The aim of the present study was to investigate whether bacterial species of the intestinal tract and other sources could interfere with P-ANCA in sera from patients with UC. METHODS: Seventeen P-ANCA-positive and anti-MPO-negative serum samples from patients with UC were tested with Escherichia coli 014 and Staphylococcus aureus Wood 46. Six of these serum samples with different P-ANCA titres were selected to test further the influence of 15 different gram-negative or gram-positive bacterial strains. Six anti-MPO positive P-ANCA, 5 anti-PR3 positive C-ANCA, and 10 antinuclear antibody (ANA)-positive serum samples were used as controls. The antineutrophil cytoplasmic antibodies (ANCAs) were analysed by an indirect immunofluorescence method (IIF) on ethanol-fixed neutrophils, and the ANAs were tested by IIF on HEp-2 cells or rat liver tissues. The bacteria used in the experiments were either live or **killed** by formalin or glutaraldehyde fixation or heated at 80 degrees C for 30 min. The test was first performed as a bacterial absorption test with sedimented organisms and then at various temperatures with the supernatant from suspension of live bacteria. RESULTS: Both MPO-positive and MPO-negative P-ANCA reactivity was abolished by absorption of patient sera with live **E. coli** and Proteus mirabilis but not with bacteria representing members of 10 other species, suggesting that antibody reactivity was absorbed away. However, continued experiments indicated that the inhibition of P-ANCA was not due to classic antigen-antibody interactions but rather to decomposition of the antigenic substrate of the neutrophils by factors present in the supernatants of live **E. coli** and P. mirabilis. The activity of the supernatant was temperature-dependent, with strong activity at room temperature and 37 degrees C, no activity at 0 degrees C, and abolished by mild heat treatment (56 degrees or 60 degrees C). No activity was shown in the supernatants from bacteria treated with **formaldehyde** or glutaraldehyde. CONCLUSIONS: Soluble material from live **E. coli** and P. mirabilis has the capacity to decompose the antigenic substrate of neutrophils responsible for both MPO-positive and MPO-negative P-ANCA, most probably brought about through enzymatic activity. Anti PR3-positive C-ANCA were not affected, which suggests substrate specificity of the proposed enzymatic activity.

L35 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1998:303526 Document No. 129:107734 Endotoxin-effects of vaccination with Escherichia coli vaccines in the pig. Garcia, P.; Hakt, H.; Magnusson, U.; Kindahl, H. (Department of Obstetrics and Gynaecology and Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Swed.). Acta Veterinaria Scandinavica, 39(1), 135-140 (English) 1998. CODEN: AVSCA7. ISSN: 0044-605X. Publisher: Acta Veterinaria Scandinavica.
AB The purpose of this study was to evaluate blood chemical and clin. response

of castrated young boars to com. available vaccines to **E. coli** where the bacteria have been **formaldehyde killed** and the endotoxins have not been removed. The animals that received the vaccine strictly s.c. did not show any clin. or blood biochem. changes as compared to a pig, which received the same dose i.v. Under clin. field circumstances the vaccinations are performed s.c./i.m. and the uptake from the injection site can vary. However, there is a risk of the vaccine coming directly into the circulation through small blood vessels. Since the boars received the same dose of the vaccines as recommended for pregnant gilts or sows in late pregnancy, the findings were discussed in terms of the risks that might be seen in pregnancy.

L35 ANSWER 6 OF 19 MEDLINE on STN

1998420426. PubMed ID: 9749978. Balance of proinflammatory and antiinflammatory cytokines in mice immunized with Escherichia coli and correlation with mortality after lethal challenge. Raponi G; Ghezzi M C; Lun M T; Mancini C. (I Chair of Clinical Microbiology, Faculty of Medicine, La Sapienza University of Rome, Italy.) Medical microbiology and immunology, (1998 Jun) Vol. 187, No. 1, pp. 11-6. Journal code: 0314524. ISSN: 0300-8584. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The balance of proinflammatory and antiinflammatory cytokines, their correlation with endotoxin levels and mortality rate after lethal challenge of Escherichia coli was investigated in mice immunized weekly for 8 weeks with formalin-**killed E. coli** either untreated or treated with 0.5x minimal inhibitory concentration of aztreonam. Control mice treated in parallel with saline, died within 24 h after challenge with 100x lethal dose (LD50) of viable **E. coli** O6:K-. Mice immunized with antibiotic-treated bacteria showed a significantly higher survival than mice immunized with untreated **E. coli**. Cytokines were not detected in the sera of control mice during the entire period of immunization. At 90 min after immunization, mice immunized with antibiotic-treated **E. coli** showed tumor necrosis factor-alpha (TNF-alpha) levels significantly lower and interleukin (IL)-6 levels significantly higher ($P < 0.05$) than mice immunized with untreated **E. coli**, while comparable levels of interferon-gamma (IFN-gamma were measured in both groups. TNF-alpha and IL-10 levels measured 90 min after lethal challenge correlated with the mortality rate observed in each group ($r = 0.96$ for TNF-alpha and 0.94 for IL-10). IL-6 levels correlated with survival ($r = 0.95$), while IFN-gamma serum levels did not differ in the two immunized groups, but were significantly higher than those measured in the control mice. IL-4 was detected only after challenge of mice immunized with antibiotic-treated bacteria. Comparable levels of circulating endotoxin were measured after lethal challenge in both control and immunized mice. These data showed that in the presence of a protective immune response the survival of immunized mice was correlated with an early alteration of cytokine expression pattern including enhanced secretion of IL-4, IL-6 and IFN-gamma, and reduced secretion of TNF-alpha and IL-10.

L35 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 3

97316536. PubMed ID: 9172447. A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and immunomagnetic separation. Restaino L; Frampton E W; Irbe R M; Allison D R. (R & F Laboratories, West Chicago, IL 60185, USA.) Letters in applied microbiology, (1997 May) Vol. 24, No. 5, pp. 401-4. Journal code: 8510094. ISSN: 0266-8254. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An antibody-direct epifluorescent filter technique (Ab-DEFT) detected 100% of the raw ground beef samples inoculated with Escherichia coli O157:H7 cells (0.15 cells g-1) and incubated in a prewarmed, modified buffered

peptone water (mBPW) non-selective enrichment broth for 5 h at 42 degrees C in an orbital shaking water bath (200 rev min-1). Over 50% of the microscopic fields viewed were positive (1-10 fluorescent cells field-1) in the Ab-DEFT. All positive screening results were confirmed within 24 h by subjecting 1 ml of the mBPW to the Dynabeads anti-**E. coli** O157 immunomagnetic separation procedure, followed by plating on MacConkey sorbitol agar containing 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide. At this cell concentration, 41.7% of the inoculated samples were detected by the conventional method involving a 24-h selective enrichment. Exposure to viable cells before filtration was minimized by using a 0.58% **formaldehyde** concentration for 5 min at 50 degrees C (**killed** > 4.00 logs of **E. coli** O157:H7 cells) without affecting cell fluorescence.

L35 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1996:307731 Document No. 124:340906 Method for immunization of poultry with vaccines. Takeda, Reiji; Ekino, Shigeo; Sugimori, Giichi; Nakamura, Takashi; Aoyama, Shigemi (Shionogi Seiyaku Kk, Japan). Jpn. Kokai Tokkyo Koho JP 08073377 A2 19960319 Heisei, 6 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1995-169717 19950705. PRIORITY: JP 1994-153342 19940705.
AB A method for immunization of poultry with vaccines against e.g. Escherichia coli type O2 or Brucella abortus for infection prevention involves: administration of the vaccine (**killed E. coli** type O2 or B. abortus) to the excretory tract of fetuses during hatching. E.g. **E. coli** type O2 vaccine is prepared by cultivation of **E. coli** type O2 in BHI medium at 37° for 24 h, treatment of cultured **E. coli** type O2 with 0.2% **formaldehyde** at room temperature for 48 h, suspension of the treated **E. coli** type O2 in 0.2% saline to final concentration of 1.5 X 1010 CFU/mL, and finally sonication.

L35 ANSWER 9 OF 19 MEDLINE on STN DUPLICATE 4
95105015. PubMed ID: 7806373. Role of endotoxin in acute inflammation induced by gram-negative bacteria: specific inhibition of lipopolysaccharide-mediated responses with an amino-terminal fragment of bactericidal/permeability-increasing protein. Kohn F R; Kung A H. (XOMA Corporation, Berkeley, California 94710.) Infection and immunity, (1995 Jan) Vol. 63, No. 1, pp. 333-9. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB A recombinant 23-kDa amino-terminal fragment of human bactericidal/permeability-increasing protein (rBPI23), a potent lipopolysaccharide (LPS)-binding/neutralizing protein, was used as a probe to assess the role of endotoxin in the acute inflammatory responses elicited by gram-negative bacteria in rat subcutaneous air pouches. In initial experiments, rBPI23 prevented the Escherichia coli O111:B4 LPS-induced accumulation of polymorphonuclear leukocytes (PMN), tumor necrosis factor alpha (TNF-alpha), and nitrite (a stable end product of nitric oxide formation) in exudate fluids. Significant inhibition of TNF-alpha production was still evident when rBPI23 treatment was delayed for 30 min after LPS instillation. In subsequent experiments, rBPI23 also prevented the nitrite and early (2-h) TNF-alpha accumulation induced by three different strains of **formaldehyde-killed** gram-negative bacteria (**E. coli** O7:K1, **E. coli** O111:B4, and Pseudomonas aeruginosa 12.4.4) but did not inhibit the PMN or late (6-h) TNF-alpha accumulation induced by these bacteria. As with LPS challenge, a significant inhibition of early TNF-alpha production was still evident when rBPI23 treatment was delayed for 30 to 60 min after instillation of **killed** bacteria. The results indicate that in this experimental model the NO and early TNF-alpha responses to gram-negative bacterial challenge are mediated predominantly by endotoxin, whereas the PMN and late TNF-alpha responses may be mediated by other bacterial components. Moreover, the results

indicate that rBPI23 can inhibit the bacterially induced production of certain potentially harmful mediators (TNF-alpha and NO) without entirely blocking the host defense, i.e., PMN response, against the bacteria.

L35 ANSWER 10 OF 19 MEDLINE on STN
96072457. PubMed ID: 8568283. In vivo chemoactivation of oyster hemocytes induced by bacterial secretion products. Alvarez M R; Friedl F E; Roman F R. (Department of Biology, University of South Florida, Tampa 33620-5150, USA.) Journal of invertebrate pathology, (1995 Nov) Vol. 66, No. 3, pp. 287-92. Journal code: 0014067. ISSN: 0022-2011. Pub. country: United States. Language: English.
AB Movements of tissue hemocytes in the Eastern oyster Crassostrea virginica were monitored and quantified by image analysis of sections following inoculation with agar cores containing Escherichia coli or cell-free medium on which the bacteria had previously grown. Hemocytes respond to the presence of live bacteria by accumulating in widely dispersed areas of tissue surrounding the gut and digestive diverticula. The response is rapid and evident within 40 min, is maximal at 1 hr, and declines by 3 hr after inoculation. Sterile implanted agar cores do not produce a response. Bacteria **killed** with ozone elicit a response when inoculated together with the medium on which they had grown while bacteria **killed** by heat or formalin do not. **Killed** bacteria suspended in saline fail to stimulate hemocyte chemokinesis. Cell-free medium applied externally produces a response equal to that measured with live bacteria inoculated internally. Extraction of bacteria-free medium with hexane does not significantly reduce hemocyte chemokinesis. Digestion of bacteria-free medium with pronase completely eliminates chemokinesis. Molecular filtrates of bacteria-free medium induce maximal chemokinetic response at molecular weight as low as 1 kDa. These data show that the oyster hemocyte activators produced by **E. coli** are most likely low-molecular-weight polypeptides which diffuse from the site of inoculation and can pass through the intact external surface epithelium to induce a chemokinetic response.

L35 ANSWER 11 OF 19 MEDLINE on STN
95282476. PubMed ID: 7762266. Vaccination with a formalin-**killed** P-fimbriated **E. coli** whole-cell vaccine prevents renal scarring from pyelonephritis in the non-human primate. Roberts J A; Kaack M B; Baskin G; Svenson S B. (Department of Urology, Tulane University School of Medicine, New Orleans, LA 70112, USA.) Vaccine, (1995 Jan) Vol. 13, No. 1, pp. 11-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB A formalin-**killed** P-fimbriated Escherichia coli serotype O4 vaccine was evaluated for protective efficacy in monkeys in an experimental pyelonephritis model following urethral bacterial inoculation. The vaccination did not protect against initial colonization and there were no significant differences in the time of bacteriuria after experimental infection in the two groups of animals. The whole-cell vaccine offers a limited protection against renal dysfunction and scarring ($p = 0.002$) and less renal involvement ($p = 0.04$), results that are quite similar to those given by a synthetic O-antigen-specific saccharide-protein conjugate vaccine previously tested.

L35 ANSWER 12 OF 19 EMBASE
92219624 EMBASE Document No.: 1992219624. Oral vaccination of weaned rabbits against enteropathogenic Escherichia coli-like **E. coli** O103 infection: Use of heterologous strains harboring lipopolysaccharide or adhesin of pathogenic strains. Milon A.; Esslinger J.; Camguilhem R.. Departement de Biologie Moleculaire, Un. Associee Microbiol. Moleculaire, INRA, 23, Chemin des Capelles,F-31076 Toulouse Cedex, France. Infection and Immunity Vol. 60, No. 7, pp. 2702-2709 1992.

ISSN: 0019-9567. CODEN: INFIBR
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 920816. Last Updated on STN: 920816

AB To test the importance of lipopolysaccharide (LPS) and adhesin as major antigens in vaccination against rabbit enteropathogenic Escherichia coli (EPEC)-like **E. coli** O103 infection, we used two nonpathogenic wild-type strains to immunize rabbits at weaning. One of these strains (C127) harbors the O103 LPS but does not express the 32,000-molecular-weight adhesin that characterizes the highly pathogenic O103 strains. The other (C6) belongs to the O128 serogroup, which does not cross-react with the O103 serogroup, but expresses the adhesin. These strains were administered orally, either live or after Formalin inactivation. After vaccination, the animals were challenged with highly pathogenic O103 strain B10. Compared with rabbits vaccinated with the Formalin-**killed** homologous strain, rabbits vaccinated with **killed** C127 or C6 showed partial but significant protection. When given live, these strains colonized more or less heavily the digestive tract of the animals and provided nearly complete (C127) or complete (C6) protection against challenge. They induced a quick local immune response, as judged by fecal immunoglobulin A anti-LPS kinetics. Furthermore, strain C6 induced an ecological effect of 'resistance to colonization' against challenge strain B10. This effect may have been due to the adhesin that is shared by both strains and to the production of a colicin. Strain C6 could inhibit in vitro the growth of highly pathogenic O103 strains. On the whole, our results show that adhesins and LPS are important, although probably not exclusive, protection-inducing components in rabbit EPEC-like colibacillosis and provide insight into possible protection of rabbits against EPEC-like **E. coli** infection with live strains.

L35 ANSWER 13 OF 19 MEDLINE on STN DUPLICATE 5

92189657. PubMed ID: 1799394. [Immunomodulating effect of **killed** , apathogenic Escherichia coli, strain Nissle 1917, on the macrophage system]. Immunmodulierende Wirkung von abgetoteten apathogenen Escherichia coli, Stamm Nissle 1917, auf das Makrophagensystem. Hockertz S. (Fraunhofer Institut fur Toxikologie, Abteilung Immunbiologie/Immunotoxikologie, Hannover.) Arzneimittel-Forschung, (1991 Oct) Vol. 41, No. 10, pp. 1108-12. Journal code: 0372660. ISSN: 0004-4172. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.

AB The influence of **formaldehyde-killed** Escherichia coli strain Nissle 1917 (SK 22) on macrophages of C57BL/6 mice was investigated in vitro. It has been shown that SK 22 activated macrophages derived from bone marrow produced Interleukin-6 with high efficiency. In addition, SK 22 stimulated macrophages to secrete tumor necrosis factor, as measured by a bioassay. Furthermore, macrophages were activated by SK 22 to produce a 3 fold amount of oxygen radicals compared to the spontaneous oxygen radical production. In contrast to this finding, the phagocytic capacity of these macrophages was only slightly increased. The specific lysis of P 815 tumor cells by peritoneal macrophages after coincubation with SK 22 was measured using tumor cells prelabelled with radioactive 51Cr. The results of the in vitro experiments presented clearly show that the **E. coli** preparation SK 22 is an efficient immunomodulator of the unspecific immune system.

L35 ANSWER 14 OF 19 MEDLINE on STN

85055122. PubMed ID: 6501409. Legionella pneumophila inhibits acidification of its phagosome in human monocytes. Horwitz M A; Maxfield F R. The Journal of cell biology, (1984 Dec) Vol. 99, No. 6, pp. 1936-43. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB We used quantitative fluorescence microscopy to measure the pH of

phagosomes in human monocytes that contain virulent Legionella pneumophila, a bacterial pathogen that multiplies intracellularly in these phagocytes. The mean pH of phagosomes that contain live L. pneumophila was 6.1 in 14 experiments. In the same experiments, the mean pH of phagosomes containing dead L. pneumophila averaged 0.8 pH units lower than the mean pH of phagosomes containing live L. pneumophila, a difference that was highly significant (P less than 0.01 in all 14 experiments). In contrast, the mean pH of phagosomes initially containing live **E. coli**, which were then **killed** by monocytes, was the same as for phagosomes initially containing dead **E. coli**. The mean pH of L. pneumophila phagosomes in activated monocytes, which inhibit L. pneumophila intracellular multiplication, was the same as in nonactivated monocytes. To simultaneously measure the pH of different phagosomes within the same monocyte, we digitized and analyzed fluorescence images of monocytes that contained both live L. pneumophila and sheep erythrocytes. Within the same monocyte, live L. pneumophila phagosomes had a pH of approximately 6.1 and sheep erythrocyte phagosomes had a pH of approximately 5.0 or below. This study demonstrates that L. pneumophila is capable of modifying the pH of its phagocytic vacuole. This capability may be critical to the intracellular survival and multiplication of this and other intracellular pathogens.

L35 ANSWER 15 OF 19 MEDLINE on STN DUPLICATE 6

84238363. PubMed ID: 6376357. In vitro cytotoxic effect of alpha-hemolytic Escherichia coli on human blood granulocytes. Gadeberg O V; Orskov I. Infection and immunity, (1984 Jul) Vol. 45, No. 1, pp. 255-60. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The cytotoxic effect of Escherichia coli bacteria on human blood granulocytes was measured by recording numbers of nonlysed cells and percentages of viable cells after in vitro incubation with bacteria in the presence of plasma. A total of 179 strains from various sources of infection were tested. Of 117 alpha-hemolytic strains, 59 were cytotoxic. Five nonhemolytic mutant strains, derived from alpha-hemolytic cytotoxic strains, were nontoxic. None of the 62 nonhemolytic strains were toxic. Four spontaneously occurring alpha-hemolytic, nontoxic mutant strains were isolated from cytotoxic ones. Cytotoxicity of bacteria reached a maximum after log-phase growth at 30 to 37 degrees C for 2.5 h, and the toxic capacity was equal after growth in various media, including human urine and plasma. The cytotoxic effect increased with the length of exposure of granulocytes to bacteria and with increasing numbers of bacteria per granulocyte. Cytotoxic strains showed different degrees of toxicity, highly cytotoxic strains lysing about 90% of the granulocytes and killing about one-half of nonlysed cells in 1 h. Bacteria **killed** by heat, **formaldehyde**, or UV light were nontoxic. Alpha-hemolytic strains of O groups 2, 4, 6, 25, and 75 originating from various infections in humans were more frequently cytotoxic than alpha-hemolytic strains of other O groups derived from human infections. Culture supernatants containing free alpha-hemolysin were highly cytotoxic to human blood granulocytes, monocytes, and lymphocytes in vitro, whether supernatants originated from cytotoxic or noncytotoxic bacteria. Cytotoxicity to phagocytes, which is mediated by or closely linked genetically to alpha-hemolysin, may be a mechanism by which alpha-hemolytic strains of **E. coli** strengthen their ability to establish and maintain infections.

L35 ANSWER 16 OF 19 MEDLINE on STN DUPLICATE 7

83056932. PubMed ID: 6815187. The role of phospholipase A2 lysines in phospholipolysis of Escherichia coli **killed** by a membrane-active neutrophil protein. Forst S; Weiss J; Elsbach P. The Journal of biological chemistry, (1982 Dec 10) Vol. 257, No. 23, pp. 14055-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Purified rabbit bactericidal/permeability-increasing protein at bactericidal concentrations is a membrane-perturbing agent that triggers hydrolysis of envelope phospholipids of a phospholipase A-less Escherichia coli (S17) mutant by a highly basic (pI greater than 10) phospholipase A2, purified from Agkistrodon halys blomhoffii snake venom. Most other purified phospholipases A2 do not degrade the phospholipids of **E. coli killed** by the bactericidal protein. To study the role of enzyme charge in bactericidal protein-dependent phospholipid hydrolysis, lysines of the Agkistrodon phospholipase A2 were modified, either by carbamylation (decreases net charge), or by reductive methylation (no delta charge). Incorporation of [14C]cyanate or [14C] **formaldehyde** and amino acid analysis served to monitor modification. Modification appears to be limited to epsilon-NH2 groups. Incorporation of up to 5 mol of cyanate or **formaldehyde**/mol of enzyme did not affect catalytic activity. In contrast, incorporation of, on average, 1 mol of either reagent/mol of protein reduced by 80% the activity of the enzyme toward **E. coli** S17 **killed** by the bactericidal protein. Since this loss is similar with carbamylation and reductive methylation, the role of the epsilon-NH2 group in the bactericidal protein-dependent hydrolysis seems independent of charge. Thus, the lysines in this phospholipase A2 are not essential for catalysis and substrate binding, but are essential for the action of this enzyme on **E. coli killed** by the bactericidal protein.

L35 ANSWER 17 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

1982:147297 Document No.: PREV198273007281; BA73:7281. LEAKAGE INDUCED IN ESCHERICHIA-COLI CELLS BY A PROTEIN RNA COMPLEXES FROM BACTERIO PHAGE F-2. DE MARS CODY J [Reprint author]; CONWAY T W. DEP BIOCHEM, UNIV IOWA, IOWA CITY, IOWA 52242, USA. Journal of Virology, (1981) Vol. 39, No. 1, pp. 60-66.
CODEN: JOVIAM. ISSN: 0022-538X. Language: ENGLISH.

AB Complexes of f2 phage RNA and its A protein, or maturation protein, transfect **E. coli** cells much better than protein-free RNA. These complexes were used to introduce the bactgeriophage f2 lysis gene into cells. The A protein-RNA complex **killed** cells, probably by causing them to leak large macromolecules. Previously induced β-galactosidase leaked from cells treated either with the A protein-RNA complex or with lethal but noninfectious complexes that had been treated with **formaldehyde.** This observation was consistent with an earlier finding that **formaldehyde**-treated f2 RNA stimulates the in vitro synthesis of a lysis protein. The complexes did not stimulate the rate of leakage of β-galactosidase from a streptomycin-resistant mutant know to be lysis defective. The rate of leakage was increased in a double mutant resistant to both streptomycin and rifampin and which is lysed normally by f2 bacteriophage.

L35 ANSWER 18 OF 19 MEDLINE on STN

81152810. PubMed ID: 7010560. Protective effect of immunization with Salmonella minnesota Re 595 against ascending Escherichia coli O6K13H1 pyelonephritis in rats. Larsson P; Kaijser B; Baltzer I M; Olling S. Scandinavian journal of infectious diseases. Supplementum, (1980) Vol. Suppl 24, pp. 220-3. Journal code: 0251025. ISSN: 0300-8878. Pub. country: Sweden. Language: English.

AB Active as well as passive immunization with formalin-**killed** S. minnesota Re 595 bacteria protected against experimental, ascending pyelonephritis caused by **E. coli** O6K13H1 in rats. Absorption of the hyperimmune sera with Re antigen before passively given did not eliminate the protective effect. The specificity of protective antibodies is discussed.

L35 ANSWER 19 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
1978:127944 Document No.: PREV197865014944; BA65:14944. EFFECT OF HEAT ON ANTIGENICITY AND IMMUNOGENICITY OF THE ANTIGENIC DETERMINANT SHARED BY HAEMOPHILUS-INFLUENZAE TYPE B AND ESCHERICHIA-COLI K-100. WHANG H Y [Reprint author]; GOLDHAR J; NETER E. LAB BACTERIOL, CHILD HOSP, BUFFALO, NY 14222, USA. Infection and Immunity, (1977) Vol. 18, No. 1, pp. 68-72. CODEN: INFIBR. ISSN: 0019-9567. Language: ENGLISH.

AB **E. coli** K100 produces an antigenic determinant similar to or identical with the capsular antigen of H. influenzae type b. The effects of heat on the immunogenicity, erythrocyte-modifying capacity and antigenicity of this cross-reacting antigen (CRA) were studied. Immunization of rabbits with viable or **formaldehyde-killed** suspensions of **E. coli** K100, producing CRA, engendered CRA antibodies in significant titers, as demonstrated by hemagglutination of erythrocytes modified by H. influenzae type b antigen. Heating of the suspensions for 1 h at 56 or 100° C destroyed the immunogenicity of CRA, and the heated suspensions did not prime for a secondary antibody response. Supernatants of heated suspensions also were non-immunogenic. Repeated freezing and thawing of heated suspensions of **E. coli** K100 or their supernatants did not restore immunogenicity. Heat also abolished the immunogenicity of H. influenzae type b. Loss of immunogenicity of CRA of **E. coli** K100 by heat was not due to alteration of the antigenic determinant, since heated suspensions and supernatants thereof modified erythrocytes for agglutination by H. influenzae type b antiserum. The latter supernatants also inhibited hemagglutination by H. influenzae type b antibodies and absorbed the latter. Striking differences exist in the effects of heat on CRA compared to enterobacterial common antigen and lipopolysaccharide O antigen of enteric bacteria. Heating of the latter 2 antigens does not abolish their priming effect, and repeated freezing and thawing restores their immunogenicity.

=> s l11 and bleach
L36 1 L11 AND BLEACH

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L36 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2002:222954 Document No.: PREV200200222954. Lactic acid bacteria protect leaves from infection by environmental microbes. Gomez, L. [Reprint author]; Cheng, A.; Watkins, C.; Dahlen, C.; Vermeulen, C. W.. Division Ave High School, Levittown, NY, USA. Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 508. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society of Microbiology.
ISSN: 1060-2011. Language: English.

AB That tender, moist mesophyll cells remain uninfected although exposed to external microbes via a leaf's stomata prompted us to think that plants may be using the same protective strategy that mammalian portal membranes use - a coating of bacteria of that division of "lactics" called the dairy bacteria. Leaves and dairy bacteria have long been associated (silage), but what these few species of slow-growing fastidious bacteria are doing in plants has not been asked. Whole leaf squashes of surface-sterilized (**bleach**) leaves were made onto paper-covered replication blocks, and then printed onto various agars resulting in abundant growth of mixed populations. Numerous streptococci and lactobacilli were identified microscopically from colonies of different morphologies. Choosing a "universal" plant - the dandelion - we found that the bacterial subpopulations were species specific over a 150 mile range. Testing

whether these bacteria merely filled a niche, or they actively protected the leaf was done both in situ and in vitro. Blue dye mixes of **E .coli** and B.subtilis were injected horizontally into the mesophyll of several leaves, and at timed intervals, the leaves were cracked open at the blue injection lines, and swabbings were plated on MacConky agar, or briefly boiled and plated on nutrient agar. Within 60 minutes, both bacteria were **killed.** An in vitro mixed culture of "leaf bacteria" in nutrient broth was also inoculated with low numbers of **E.coli** and B.subtilis. (Filtrates were not lethal to the "contaminants.") Again within an hour both became undetectible. Thus, not only are "lactics" copiously present INSIDE leaves, but they also have the capacity, presumably via bacteriocins, to kill other microbes common in the environment. Implications: (1) evolution - two kingdoms using the same protection strategy; and (2) agriculture - development of lactics with more potent bacteriocins.

=> s l11 and ozone
L37 32 L11 AND OZONE

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L38 15 DUP REMOVE L37 (17 DUPLICATES REMOVED)

=> d l38 1-15 cbib abs

L38 ANSWER 1 OF 15 MEDLINE on STN DUPLICATE 1
2006248007. PubMed ID: 16672466. Inactivation of enterohemorrhagic Escherichia coli in rumen content- or feces-contaminated drinking water for cattle. Zhao Tong; Zhao Ping; West Joe W; Bernard John K; Cross Heath G; Doyle Michael P. (Center for Food Safety, University of Georgia, Griffin, GA 30223, USA.) Applied and environmental microbiology, (2006 May) Vol. 72, No. 5, pp. 3268-73. Journal code: 7605801. ISSN: 0099-2240. Pub. country: United States. Language: English.

AB Cattle drinking water is a source of on-farm Escherichia coli O157:H7 transmission. The antimicrobial activities of disinfectants to control **E. coli** O157:H7 in on-farm drinking water are frequently neutralized by the presence of rumen content and manure that generally contaminate the drinking water. Different chemical treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, **ozone**, butyric acid, sodium benzoate, and competing **E. coli**, were tested individually or in combination for inactivation of **E. coli** O157:H7 in the presence of rumen content. Chlorine (5 ppm), **ozone** (22 to 24 ppm at 5 degrees C), and competing **E. coli** treatment of water had minimal effects (<1 log CFU/ml reduction) on killing **E. coli** O157:H7 in the presence of rumen content at water-to-rumen content ratios of 50:1 (vol/wt) and lower. Four chemical-treatment combinations, including (i) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (treatment A); (ii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (treatment B); (iii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (treatment C); and (iv) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 100 ppm chlorine dioxide (treatment D); were highly effective (>3 log CFU/ml reduction) at 21 degrees C in killing **E . coli** O157:H7, O26:H11, and O111:NM in water heavily contaminated with rumen content (10:1 water/rumen content ratio [vol/wt]) or feces (20:1 water/feces ratio [vol/wt]). Among them, treatments A, B, and C **killed** >5 log CFU **E. coli** O157:H7, O26:H11, and O111:NM/ml within 30 min in water containing rumen content or feces, whereas treatment D inactivated approximately 3 to 4 log CFU/ml under the same conditions. Cattle given water containing treatment A or C

or untreated water (control) ad libitum for two 7-day periods drank 15.2, 13.8, and 30.3 liters/day, respectively, and cattle given water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 liters/day. The amounts of water consumed for all water treatments were significantly different from that for the control, but there were no significant differences among the water treatments. Such treatments may best be applied periodically to drinking water troughs and then flushed, rather than being added continuously, to avoid reduced water consumption by cattle.

L38 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
2006:1302956 Experimental observation on bactericidal efficacy of OM-500 **ozone** air disinfector. Luo, Jun; Long, Bei-guo; Long, Min; Lai, Jian-ping; Zhang, Wen-bing (Department of Microbiology, School of Public Health and Tropic Medicine, Southern Medical University, Guangzhou, 510515, Peop. Rep. China). Xiandai Yufang Yixue, 33(10), 1954-1955 (Chinese) 2006. CODEN: XYYIFS. ISSN: 1003-8507. Publisher: Xiandai Yufang Yixue Zazhishe.
AB Objective: To study the air disinfection effect of Om-500 **ozone** air disinfector using two methods. Methods: When carrier quant. test was used, we found that 94.41% of Staphylococcus aureus and 100% of **E. coli** was **killed** if Om-500 **ozone** air disinfector for 60 min, and 97.3% of **E. coli** was **killed** if operated for 5 min. When simulate locale air disinfection test was used, the result shows that decrease rate of nature bacterium in air can reach to 90.88% and 95.74% resp. Conclusion: The experiment suggested that it has a good air disinfection effect.

L38 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
2006:462613 Document No. 145:425652 Development of a supersonic levitation washer-disinfector using **ozone** micro-bubbling and silver electrolysis. Ueda, Toyotoshi; Hara, Masanori; Nishiyama, Kyohei; Ando, Satoru; Shimizu, Mitsuhiro; Shigihara, Takanori; Koshiba, Mamiko; Nakamura, Shun (Department of Chemistry, Faculty of Science and Engineering, Meisei University, 2-1-1, Hodokubo, Hino-shi, Tokyo, 191-8506, Japan). Bokin Bobai, 34(4), 201-209 (Japanese) 2006. CODEN: BOBODP. ISSN: 0385-5201. Publisher: Nippon Bokin Bobai Gakkai.
AB A new type of supersonic washer-disinfector using **ozone** micro-bubbling and silver electrolysis was developed in order to clean and disinfect many devices and materials such as semiconductors, endoscopes and cut vegetables. This washer has fourteen supersonic oscillators of an umbrella shape, which emit supersonic traveling waves along more than two directions and are driven independently by each supersonic transducer. This supersonic levitation washer can evenly clean not only hard materials such as glasses, jewels and metals, but also soft materials such as clothes, plastics, rubbers and bodies. Neither detergent nor disinfectant is necessary: therefore, its drainage does not cause environmental pollution. Disinfection is easy and rapid using **ozone** oxidation and silver electrolysis. **Ozone** is produced by the irradiation of UV light to the atmospheric oxygen and jets as micro-bubbles. Electrolysis is carried out using a d.c. between the pos. electrode of a net-shaped silver plate and the neg. electrode of a stainless-steel container. **E. coli** (103-106 cells/mL) was **killed** within 20 min either by **ozone** or silver electrolysis. Bacillus atrophaeus (104 cells/mL) was **killed** in 30 min by **ozone** and in 5 min by silver electrolysis. S. cerevisiae (104 cells/mL) was **killed** in 1 min by silver electrolysis. This apparatus meets the new demand for cleaning with the conservation of the global environment in mind.

L38 ANSWER 4 OF 15 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2
2004:983208 The Genuine Article (R) Number: 865BN. Electrochemical wastewater

disinfection: Identification of its principal germicidal actions. Li X Y (Reprint); Diao H F; Fan F X J; Gu J D; Ding F; Tong A S F. Univ Hong Kong, Dept Civil Engn, Pokfulam Rd, Hong Kong, Hong Kong, Peoples R China (Reprint); Univ Hong Kong, Dept Civil Engn, Hong Kong, Hong Kong, Peoples R China; Tsing Hua Univ, Dept Environm Sci & Engn, Beijing, Peoples R China; Macao Water Supply Co Ltd, SAAM, Macau, Peoples R China; Univ Hong Kong, Dept Ecol & Biodivers, Hong Kong, Hong Kong, Peoples R China; Hong Kong SAR Govt, Environm Protect Dept, Hong Kong, Hong Kong, Peoples R China. xlia@hkucc.hku.hk. JOURNAL OF ENVIRONMENTAL ENGINEERING-ASCE (OCT 2004) Vol. 130, No. 10, pp. 1217-1221. ISSN: 0733-9372. Publisher: ASCE-AMER SOC CIVIL ENGINEERS, 1801 ALEXANDER BELL DR, RESTON, VA 20191-4400 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Laboratory experiments were carried out to investigate the mechanisms of electrochemical (EC) wastewater disinfection. Artificial wastewater contaminated by Escherichia coli (**E. coli**) culture, and which contained different salts of NaCl, Na2SO4, and NaNO3, was used as the test medium. The experimental results do not favor the hypotheses that the EC bactericidal action was due to cell destruction by the electric field and the production of persulfate. In comparison to direct chlorination, the EC process displayed a much stronger disinfecting capability than that of electrochlorination assumed for EC disinfection. Observations with scanning electron microscopy on the **E. coli** bacteria of wastewater treated by different means of disinfection suggested that the cells were likely **killed** during the EC treatment by chemical products with oxidizing and germicidal powers similar to that of **ozone** and much stronger than that of chlorine. All of the findings support the theory that the major killing function of EC disinfection is provided by short-lived and high-energy intermediate EC products, such as free radicals.

L38 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
2004:598356 Document No. 142:193766 Comparison of examination of germicidal efficacy of **ozone** water by two test methods. Jiang, Li; Wang, Taixing; Rao, Lin (Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China). Zhongguo Xiaoduxue Zazhi, 20(1), 11-13 (Chinese) 2003. CODEN: ZXZAFO. ISSN: 1001-7658. Publisher: Zhongguo Xiaoduxue Zazhi Bianjibu.

AB Suspension quant. germicidal test method and quant. germicidal test method using carriers immersed in running liquid disinfectant for examining efficacy of **ozone** water in killing Escherichia coli were compared and the influence of peptone on its germicidal efficacy was examined The results indicated that when suspension quant. germicidal test method was used, in absence of peptone, the **ozone** water containing **ozone** 8.0 mg/L with a 1 min contact time **killed** 100% of **E. coli** in average and if peptone 10 g/L was present, the same **ozone** water with a 10 min contact time **killed** 65.96% of **E. coli** in average When quant. germicidal test method using carriers immersed in running liquid disinfectant was used, the **ozone** water containing **ozone** 8.0 mg/L with a 10 min contact time **killed** 99.97% of **E. coli** in average and if the bacterial suspension contained higher than 25% volume of calf serum, the germicidal efficacy was influenced significantly. The results suggest that when running **ozone** water is used in surface disinfection, examination of germicidal efficacy by quant. germicidal test method using carriers immersed in running liquid disinfectant relatively approximates the real condition.

L38 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
2002:964231 Document No. 138:44757 Medical devices treatment with **ozone** for prevention of infection. Darouiche, Rabih O.; Shannon, David C. (Baylor College of Medicine, USA). PCT Int. Appl. WO 2002100455

A2 20021219, 30 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US17806 20020605. PRIORITY: US 2001-296837P 20010608.

AB Indwelling medical devices resistant to microbial colonization and other complications include devices having a coating on 1 or more surfaces comprising an effective amount or concentration of an oxygen-releasing substance, such as **ozone**, and optionally, other therapeutic agents. Devices may alternately include a sleeve or other means which allows one or more surfaces of the device to be flushed or insufflated periodically with **ozone** or another oxygen-releasing substance. A clin. isolate of Escherichia coli strain 2131 that had caused catheter-related infection was used. In the exptl. arm, **ozone** was bubbled into the bacterial suspension. In the control arm, no **ozone** was bubbled. **Ozone killed E. coli** in solution

L38 ANSWER 7 OF 15 MEDLINE on STN DUPLICATE 3

2001335889. PubMed ID: 11403125. Inactivation of Escherichia coli O1 57:H7, Listeria monocytogenes, and Lactobacillus leichmannii by combinations of **ozone** and pulsed electric field. Unal R; Kim J G; Yousef A E. (Department of Food Science and Technology, The Ohio State University, Columbus 43210, USA.) Journal of food protection, (2001 Jun) Vol. 64, No. 6, pp. 777-82. Journal code: 7703944. ISSN: 0362-028X. Pub. country: United States. Language: English.

AB Pulsed electric field (PEF) and **ozone** technologies are nonthermal processing methods with potential applications in the food industry. This research was performed to explore the potential synergy between **ozone** and PEF treatments against selected foodborne bacteria. Cells of Lactobacillus leichmannii ATCC 4797, Escherichia coli O157:H7 ATCC 35150, and Listeria monocytogenes Scott A were suspended in 0.1% NaCl and treated with **ozone**, PEF, and **ozone** plus PEE Cells were treated with 0.25 to 1.00 microg of **ozone** per ml of cell suspension, PEF at 10 to 30 kV/cm, and selected combinations of **ozone** and PEF. Synergy between **ozone** and PEF varied with the treatment level and the bacterium treated. L. leichmannii treated with PEF (20 kV/cm) after exposure to 0.75 and 1.00 microg/ml of **ozone** was inactivated by 7.1 and 7.2 log10 CFU/ml, respectively; however, **ozone** at 0.75 and 1.00 microg/ml and PEF at 20 kV/cm inactivated 2.2, 3.6, and 1.3 log10 CFU/ml, respectively. Similarly, **ozone** at 0.5 and 0.75 microg/ml inactivated 0.5 and 1.8 log10 CFU/ml of **E. coli**, PEF at 15 kV/cm inactivated 1.8 log10 CFU/ml, and **ozone** at 0.5 and 0.75 microg/ml followed by PEF (15 kV/cm) inactivated 2.9 and 3.6 log10 CFU/ml, respectively. Populations of L. monocytogenes decreased 0.1, 0.5, 3.0, 3.9, and 0.8 log10 CFU/ml when treated with 0.25, 0.5, 0.75, and 1.0 microg/ml of **ozone** and PEF (15 kV/cm), respectively; however, when the bacterium was treated with 15 kV/cm, after exposure to 0.25, 0.5, and 0.75 microg/ml of **ozone**, 1.7, 2.0, and 3.9 log10 CFU/ml were **killed**, respectively. In conclusion, exposure of L. leichmannii, **E. coli**, and L. monocytogenes to **ozone** followed by the PEF treatment showed a synergistic bactericidal effect. This synergy was most apparent with mild doses of **ozone** against L. leichmannii.

L38 ANSWER 8 OF 15 EMBASE on STN DUPLICATE 4

2001224959 EMBASE Impairment of microbial killing and superoxide-producing activities of alveolar macrophages by a low level of **ozone**. Mochitate K.; Katagiri K.; Miura T.. K. Mochitate, Environ. Health Sciences Division, Natl. Inst. for Environ. Studies, 16-2 Onogawa,

Tsukuba, Ibaraki 305-0053, Japan. mochitat@nies.go.jp. Journal of Health Science Vol. 47, No. 3, pp. 302-309 2001.
Refs: 30.
ISSN: 1344-9702. CODEN: JHSCFD
Pub. Country: Japan. Language: English. Summary Language: English.
Entered STN: 20010717. Last Updated on STN: 20010717

AB Male Wistar rats were exposed to 0.2 ppm **ozone** for up to 14 days, during which alveolar macrophages were collected by pulmonary lavage to assess the effect of **ozone** on their microbial killing and superoxide-producing activities. For rapid assessment of microbial killing activity, we measured the release of (3)H-radioactivity into the supernatant by deoxycholate-lysis of the macrophages that had phagosytosed and **killed** (3)H-uridine-labeled microbes. The killing activity against Escherichia coli and Candida albicans was reduced to 70-80% of control levels on day 3. However, phagocytosis by and the activity of lysosomal enzymes of the macrophages were not impaired. On day 14 the killing activity against **E. coli** had returned to control levels, whereas that against C. albicans was still reduced. Because active oxygen species plays an important role in microbial killing activity of macrophages, the effects of **ozone** on respiratory burst and superoxide production were examined. Aliquots of alveolar macrophages were stimulated with phorbol myristate acetate (PMA), opsonized zymosan, or lipopolysaccharide (LPS) plus cytochalasin E (Cyt.E). The respiratory burst, oxygen consumption for rapid superoxide production, was decreased to 60-80% of control levels on day 3. On day 14, the respiratory burst by opsonized zymosan was still 80% reduced, whereas that by PMA or LPS plus Cyt.E had returned to control levels. In addition, the superoxide-producing activity of **ozone**-exposed macrophages was 10-60% decreased on day 3. On day 14, the superoxide production by stimulation with opsonized zymosan was still 60% reduced, whereas that by PMA or LPS plus Cyt.E had returned to control levels. In conclusion, because of their decreased production of superoxide, the host defense activity of alveolar macrophages was impaired by in vivo exposure to 0.2 ppm **ozone**. In particular, the C. albicans-associated defect lasted throughout the exposure period.

L38 ANSWER 9 OF 15 MEDLINE on STN

2001396497. PubMed ID: 11447890. Reviewing efficacy of alternative water treatment techniques. Hambidge A. Health estate, (2001 Jun) Vol. 55, No. 6, pp. 23-5. Journal code: 100888268. Pub. country: England: United Kingdom. Language: English.

AB This section is designed to provide a brief summary of some of the findings. A good deal of work has been conducted by Mr N. L. Pavey and the team at BSRIA, Bracknell. The BSRIA publications are an excellent source of further information. Ultraviolet radiation: UV radiation of wavelength 254 nm destroys bacteria by a mechanism of damaging nucleic acids by producing thymine dimers which disrupt DNA replication [Gavdy and Gavdy, 1980]. L. pneumophila has been reported as sensitive to UV dosages of 2,500-7,000 uW.s/cm2 [Antopol & Ellner, 1979; Knudson, 1985]. Antopol and Ellner [1979] examined the susceptibility of L. pneumophila to UV dosage. Their results indicated that 50% of the organisms were **killed** by 380 uWs/cm2 and 90% were **killed** by 920 uWs/cm2. Kills of 99 and 99.9% were obtained using 1,840 and 2,760 uWs/cm2 respectively. Muraca et al [1987] showed that continuous UV irradiation resulted in a 5 logarithm decrease in waterborne L. pneumophila in a circulating system. Gilpin [1984] reported that in laboratory buffer solutions, exposure to 1 uW of UV radiation per cm2 achieved a 50% kill of L longbeachae in 5 minutes, L. gormanii in 2-30 minutes and L pneumophila in 17 minutes. Exposure times for 99% kills for L. longbeachae, L pneumophila and L. Gormanii were 33, 48 and 63 minutes respectively. The same research worker conducted experiments using a 3 litre circulating water system, connected to a stainless steel housing

containing a UV source. The UV lamp output was 7 ergs/mm2 per second per 100 cm at 254 nm. L. pneumophila was **killed** within 15 seconds, that is within their first pass through the system. Continuous disinfection with UV has the advantages of imparting no taste, odour or harmful chemical by-products and requires minimal operation and maintenance [Muraca et al 1988]. Keevil et al [1989] state that UV irradiation fails to clear systems of biofilm because of poor penetration into microflocs of the micro-organisms. Copper/silver ionisation: A recent study of full scale hot water test rigs incorporating copper-silver ionisation systems has been reported by Pavey, 1996. Copper and silver ions were introduced into the water by electrolysis. One of the principal mechanisms of biocidal action of these ions is thought to be cell penetration. The positively charged copper ions form electrostatic bonds with negatively charged sites on the cell wall. The cell membrane is thus distorted, allowing ingress of silver ions which attack the cell by binding at specific sites to DNA, RNA, respiratory enzymes and cellular protein, causing catastrophic failure of the life support systems of the cell. Silver and copper ion concentrations of 40 and 400 ug/L respectively were effective against planktonic Legionellae in cold water systems and hot water systems containing soft water. In hard water, the ionisation was ineffective due to the inability to control silver ion concentrations. This was caused by scaling of the electrodes and silver ion complexation by the high concentration of dissolved solids. Bosch et al [1993] had earlier extended the application of copper-silver disinfection to human enteric viruses in water, such as adenovirus, rotavirus, hepatitis A virus, and poliovirus. Their work showed that copper and silver ions in the presence of reduced levels of free chlorine did not ensure the total elimination of viral pathogens from water. In the case of an amoeba, Naegleria fowleria [responsible for primary amoebic meningoencephalitis], Cassells et al [1995] have demonstrated that a combination of silver and copper ions were ineffective at inactivating the amoebae at 80 and 800 ug/L respectively. However addition of 1.0 mg/L free chlorine produced a synergistic effect, with superior inactivation relative to either chlorine or silver-copper in isolation. A similar synergy was reported by Yahya et al [1989] in their study of Staphylococcus sp. and Pseudomonas aeruginosa. Yahya et al [1992] also suggested an additive or synergistic effect in the inactivation of coliphage MS-2 and poliovirus. Other techniques: There are a number of other techniques. We have conducted trials of most of these in the control of Legionella sp., but these fall out of the scope of this article, and as such less emphasis has been placed on them here. Ozonation: **Ozone** [O3] is an oxidising gas, generated electrically from oxygen [O2]. L. pneumophila can be **killed** at < 1 mg/L of **ozone** [Edelstien et al 1982]. Muraca et al [1987] found that 1-2 mg/L of continuous **ozone** over a six hour contact time, produced a 5 logarithm decrease of L. pneumophila. The effectiveness of **ozone** treatment against a range of bacteria and coliphages has been studied Botzenhart et al [1993]. **E. coli** was least resistant to **ozone**, followed by MS 2-coliphage and PhiX 174-coliphage, with L. pneumophila and Bacillus subtilis spores being the most resistant. (ABSTRACT TRUNCATED)

L38 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
1999:347130 Document No. 131:184089 Disinfection of fresh vegetables by various means and the bactericidal effects of strong acidic electrolyzed solution on enteropathogenic bacteria. Ueda, Shigeko; Kuwabara, Yoshihiro (Hygiene Laboratory, Kagawa Nutrition University, Saitama, Sakado-shi, Chiyoda, 350-0214, Japan). Bokin Bobai, 27(5), 301-307 (Japanese) 1999. CODEN: BOBODP. ISSN: 0385-5201. Publisher: Nippon Bokin Bobai Gakkai.
AB Five kinds of fresh salad vegetables such as parsley, sprouts, sani-lettuce, cabbage and cucumber were washed and disinfected for 5 min in various disinfectants and detergents including strong or weak acidic

electrolyzing solns., **ozone**-water, hypochlorite solution, acetic acid solution, com. detergents and so on. Total aerobic bacterial counts of vegetables were depressed more effectively by these treatments than by washing only with tap water. Particularly, the treatment with a strong acidic electrolyzing solution decreased the bacterial counts by about 10-2cfu/g on all vegetables. Furthermore, among 17 kinds of vegetables treated with strong acidic electrolyzed solution, cabbage, sani-lettuce, lettuce, spinach and parsley had decreases in bacterial counts in the ranges of 10-2-103cuff/g. Similarly, coliforms, faecal **E. coli** and B. cereus were shown to decrease in number on all of vegetables after the treatment. The bacterial effects of strong acidic electrolyzing solns. and hypochlorite solns. with the same levels of active chlorine on various types of bacterial were examined Although gram neg. bacterial were **killed** within 3 min and staphylococci were completely **killed** within 30 s after exposure to the acidic electrolyzing solution, the time needed to kill spore-forming bacteria was more than 5 min. The bactericidal activity of strong acidic electrolyzing solns. was shown to be relatively higher than that of hypochlorite solns.

L38 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
1999:502120 Document No. 132:89295 Experimental observation on germicidal efficacy of TT-100-type **ozone** disinfector. Liao, Ruyan; Lin, Jinyan; Chen, Wensheng; Huang, Xianzhong; Chen, Hongmin (Guangdong Provincial Sanitary and Anti-Epidemic Station, Canton, 510300, Peop. Rep. China). Zhongguo Xiaoduxue Zazhi, 16(2), 84-87 (Chinese) 1999. CODEN: ZXZAFO. ISSN: 1001-7658. Publisher: Zhongguo Xiaoduxue Zazhi Bianjibu.
AB TT-100-type **Ozone** Disinfector generates **ozone** 1.50-1.62 mg/min in average The killing rates of Escherichia coli and Staphylococcus aureus in artificially contaminated water were 100% after introduction of **ozone** generated by operation of the disinfector for 5 min and 10 min into the water resp. The killing rate of Bacillus subtilis var. niger spores in water was only 81.23% after introduction of **ozone** for 30 min. Immersion of bacteria carriers made by different materials and contaminated with **E. coli** or S. aureus in water which was then treated with introduction of **ozone** for 15 min **killed** more than 99.9% of the bacteria on surfaces of aluminum and glass carriers and less than 99.9% of the bacteria on cloth and paper carriers. Natural bacteria test indicated that immersion of spinach and apples in water which was then treated with introduction of **ozone** for 15 min **killed** more than 94.9% of the natural bacteria on their surfaces. Introduction of **ozone** generated by disinfector for 15 min into a 18 m3 unoccupied room could reduce the total air bacteria count by 83.83%.

L38 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 5
96035678. PubMed ID: 7574656. Efficacy of ozonated water against various food-related microorganisms. Restaino L; Frampton E W; Hemphill J B; Palnikar P. (R & F Laboratories, Inc., Bridgeview, Illinois 60455, USA.) Applied and environmental microbiology, (1995 Sep) Vol. 61, No. 9, pp. 3471-5. Journal code: 7605801. ISSN: 0099-2240. Pub. country: United States. Language: English.
AB The antimicrobial effects of ozonated water in a recirculating concurrent reactor were evaluated against four gram-positive and four gram-negative bacteria, two yeasts, and spores of Aspergillus niger. More than 5 log units each of Salmonella typhimurium and Escherichia coli cells were **killed** instantaneously in ozonated water with or without addition of 20 ppm of soluble starch (SS). In ozonated water, death rates among the gram-negative bacteria--S. typhimurium, **E. coli**, Pseudomonas aeruginosa, and Yersinia enterocolitica--were not significantly different (P > 0.05). Among gram-positive bacteria, Listeria monocytogenes was significantly P < 0.05) more sensitive than either Staphylococcus aureus or Enterococcus faecalis. In the presence of

organic material, death rates of S. aureus compared with L. monocytogenes and **E. coli** compared with S. typhimurium in ozonated water were not significantly ($P > 0.05$) affected by SS addition but were significantly reduced ($P < 0.05$) by addition of 20 ppm of bovine serum albumin (BSA). More than 4.5 log units each of Candida albicans and Zygosaccharomyces bailii cells were **killed** instantaneously in ozonated water, whereas less than 1 log unit of Aspergillus niger spores was **killed** after a 5-min exposure. The average **ozone** output levels in the deionized water (0.188 mg/ml) or water with SS (0.198 mg/ml) did not differ significantly ($P < 0.05$) but were significantly lower in water containing BSA (0.149 mg/ml).

L38 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 6
96072457. PubMed ID: 8568283. In vivo chemoactivation of oyster hemocytes induced by bacterial secretion products. Alvarez M R; Friedl F E; Roman F R. (Department of Biology, University of South Florida, Tampa 33620-5150, USA.) Journal of invertebrate pathology, (1995 Nov) Vol. 66, No. 3, pp. 287-92. Journal code: 0014067. ISSN: 0022-2011. Pub. country: United States. Language: English.

AB Movements of tissue hemocytes in the Eastern oyster Crassostrea virginica were monitored and quantified by image analysis of sections following inoculation with agar cores containing Escherichia coli or cell-free medium on which the bacteria had previously grown. Hemocytes respond to the presence of live bacteria by accumulating in widely dispersed areas of tissue surrounding the gut and digestive diverticula. The response is rapid and evident within 40 min, is maximal at 1 hr, and declines by 3 hr after inoculation. Sterile implanted agar cores do not produce a response. Bacteria **killed** with **ozone** elicit a response when inoculated together with the medium on which they had grown while bacteria **killed** by heat or formalin do not. **Killed** bacteria suspended in saline fail to stimulate hemocyte chemokinesis. Cell-free medium applied externally produces a response equal to that measured with live bacteria inoculated internally. Extraction of bacteria-free medium with hexane does not significantly reduce hemocyte chemokinesis. Digestion of bacteria-free medium with pronase completely eliminates chemokinesis. Molecular filtrates of bacteria-free medium induce maximal chemokinetic response at molecular weight as low as 1 kDa. These data show that the oyster hemocyte activators produced by **E. coli** are most likely low-molecular-weight polypeptides which diffuse from the site of inoculation and can pass through the intact external surface epithelium to induce a chemokinetic response.

L38 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
1992:423156 Document No. 117:23156 Growth delay and inactivation of intracellular catalase of resting Escherichia coli K-12 cells exposed to **ozone**. Shimada, Keiko; Takahashi, Minako; Shimahara, Kenzo (Eng. Coll., Seikei Univ., Tokyo, Japan). Seikei Daigaku Kogakubu Kogaku Hokoku, 53, 3607-8 (Japanese) 1992. CODEN: SKKGAW. ISSN: 0582-4184.

AB An **E. coli** culture in a 100 μM **ozone** solution lost .apprx.90% of its catalase activity and >99% of the cells were **killed**. Repeated exposure (5 min each) of the cells to fresh solns. of **ozone** resulted in more extensive killing than a single exposure.

L38 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
1963:54974 Document No. 58:54974 Original Reference No. 58:9438a-c Effect of **ozone** on survival and permeability of Escherichia coli. McNair Scott, D. B.; Lesher, E. C. (Univ. of Pennsylvania, Philadelphia). Journal of Bacteriology, 85, 567-76 (Unavailable) 1963. CODEN: JOBAAY. ISSN: 0021-9193.

AB **E. coli** cultures in the logarithmic phase or resting

were treated with various concns. of **ozone** in saline solution Approx. 2 + 107 mols. of **ozone** per bacterium **killed** 50% of the cells. **Ozone** caused leakage of cell content into the medium, and lysis of some cells. Low concns. did not react with the glutathione within the cells, although reaction with glutathione in solution was immediate and stoichiometric. The effect on nucleic acid within the cells was to change the solubility and to cause the release of ultraviolet-absorbing material into the medium. **Ozone** attacked the ring structure of the base or the carbohydrate only when the substance was in the medium. Nucleic acids released into the medium were reabsorbed by cells which were not, lysed. Viable cells resumed growth immediately, and grew at rates determined by the nutrients either added to the medium or which resulted from leakage and lysis of nonviable cells. It is postulated that the primary attack of **ozone** was on the cell wall or membrane of the bacteria, probably by reaction with the double bonds of lipids, and that leakage or lysis of the cells depended on the extent of that reaction.

=> s l11 adn alcohol
MISSING OPERATOR L11 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l11 and alcohol
L39 29 L11 AND ALCOHOL

=> dup remove l39
PROCESSING COMPLETED FOR L39
L40 19 DUP REMOVE L39 (10 DUPLICATES REMOVED)

=> d l40 1-19 cbib abs

L40 ANSWER 1 OF 19 MEDLINE on STN DUPLICATE 1
2003003037. PubMed ID: 12475283. Green-leaf-derived C6-aroma compounds with potent antibacterial action that act on both Gram-negative and Gram-positive bacteria. Nakamura Soichiro; Hatanaka Akikazu. (Department of Life and Environmental Sciences, Shimane University, Shimane 690-8550,. Japan.soichiro@edu.shimane-u.ac.jp) . Journal of agricultural and food chemistry, (2002 Dec 18) Vol. 50, No. 26, pp. 7639-44. Journal code: 0374755. ISSN: 0021-8561. Pub. country: United States. Language: English.
AB All eight C6-aliphatic **alcohol** and aldehyde compounds in naturally occurring green leaves showed bacteriostatic effects against Staphylococcus aureus IFO 12732, methicillin-resistant S. aureus, Escherichia coli IFO 3301, **E. coli** O157:H7, and Salmonella enteritidis, with bacteriostatic activities of less than 12.5 microg mL(-1). In this study, the susceptibility of Gram-positive bacteria tested was observed to be greater than that of Gram-negative bacteria. The bactericidal action of the aldehyde compounds was found to be much stronger than that of the **alcohol** compounds under both liquid and gaseous conditions. The most effective compound was (3E)-hexenal at concentrations of 0.1 and 1 microg mL(-1), which **killed** 2.1 x 10(5) cfu mL(-1) of S. aureus IFO 12732 and 1.4 x 10(5) cfu mL(-1) of **E. coli** IFO 3301, respectively, by direct contact with the compound. Lethality of (3E)-hexenal against S. aureus IFO 12732 and **E. coli** IFO 3301 was also observed as a result of gaseous contact at concentrations of 3 and 30 microg mL(-1), respectively. The bactericidal effects of 30 microg mL(-1) (3E)-hexenal were thoroughly maintained throughout periods of 2 days and 1 day against S. aureus IFO 12732 and **E. coli** IFO 3301, respectively, by a complex formation with alpha-cyclodextrin.

L40 ANSWER 2 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2002:369022 The Genuine Article (R) Number: 543LZ. Liver sinusoidal endothelial cell injury by neutrophils in rats with acute obstructive cholangitis. Gong J P (Reprint); Wu C X; Liu C A; Li S W; Shi Y J; Li X H; Peng Y. Chongqing Univ Med Sci, Coll Clin Med 2, Dept Gen Surg, 74 Linjuang Rd, Chongqing 400010, Peoples R China (Reprint); Chongqing Univ Med Sci, Coll Clin Med 2, Dept Gen Surg, Chongqing 400010, Peoples R China; Chongqing Univ Med Sci, Affiliated Hosp 2, Chongqing 400010, Peoples R China. WORLD JOURNAL OF GASTROENTEROLOGY (APR 2002) Vol. 8, No. 2, pp. 342-345. ISSN: 1007-9327. Publisher: W J G PRESS, PO BOX 2345, BEIJING 100023, PEOPLES R CHINA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB AMI: The objective of this study is to elucidate the potential role of poly-morphonuclear neutrophils (PMN) in the development of such a sinusoidal endothelial cell (SEC) injury during early acute obstructive cholangitis (AOC) in rats.

METHODS: Twenty one Wistar rats were divided into three groups: the ACC group. the bile duct ligated group (BDL group), and the sham operation group (SO group), The common bile duct (CBD) of rats in ACC group was dually ligated and 0. 2 ml of the **E. coli** O-111 B-4 (5 x 10(9) cfu/ml) suspension was injected into the upper segment, in BDL group, only the CBD was ligated and in SO group. neither injection of **E. coli** suspension nor CBD ligation was done. but the same operative procedure. Such group consisted of seven rats, all animals were **killed** 6 h after the operation. Morphological changes of the liver were observed under light and electron microscope. Expression of intercellular adhesion molecule-1 (ICAM-1) mRNA in hepatic tissue was determined with reverse transcription polymerase chain reaction (RT-PCR). The serum levels of alanine aminotransferase (ALT) were determined with anutoanalyger and cytokine-induced neutrophil chemoattractant (CINC) was determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS: Neutrophils was accumulated in the hepatic sinusoids and sinusoidal endothelial cell injury existed in ACC group. In contrast, in rats of BDL group, all the features of SEC damage were greatly reduced, Expression of ICAM-1 mRNA in hepatic tissue in three groups were 7.54 +/- 0, 82, 2. 87 +/- 0. 34, and 1. 01 +/- 0. 12, respectively. There were significant differences among three groups P < 0. 05). The serum CINC levels in the three groups were 188 +/- 21 ng . L-1, 94 +/- 11 ng . L-1, and 57 +/- 8 ng . L-1, respectively. There were also significant differences among the three groups (P < 0. 05). Activity of the serum ALT was 917 &PLUSMN; 167 nkat &BULL; L-1. 901 &PLUSMN; 171 nkat &BULL; L-1. and 908 &PLUSMN; 164 nkat &BULL; L-1. respectively, (P > 0.005).

CONCLUSION: Hepatic SEC injury occurs earlier than hepatic parenchymal cells during AOC. Recruitments of circulating neutrophils in the hepatic sinusoidal space might mediate the SEC injury, and ICAM-1 in the liver may modulate the PMN of accumulation.

L40 ANSWER 3 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2001:421969 The Genuine Article (R) Number: 431MG. Wine has activity against entero-pathogenic bacteria in vitro but not in vivo. Sugita-Konishi Y (Reprint); Hara-Kudo Y; Iwamoto T; Kondo K. Natl Inst Infect Dis, Dept Biomed Food Res, Shinjuku Ku, 1-23-1 Toyama, Tokyo 1628640, Japan (Reprint); Natl Inst Infect Dis, Dept Biomed Food Res, Shinjuku Ku, Tokyo 1628640, Japan; Natl Inst Hlth & Nutr, Dept Food Sci, Shinjuku Ku, Tokyo 1628640, Japan; Ochanomizu Univ, Fac Human Life & Environm Sci, Bunkyou Ku, Tokyo 1128610, Japan. BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY (APR 2001) Vol. 65, No. 4, pp. 954-957. ISSN: 0916-8451. Publisher: JAPAN SOC BIOSCI BIOTECHN AGROCHEM, JAPAN ACAD SOC CTR BLDG, 2-4-6 YAYOI BUNKYO-KU, TOKYO, 113, JAPAN. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We studied the activity of wine against entero-pathogenic bacteria both lit vitro and in vivo, The food-borne bacteria were **killed** in both red and white wine within 30 min. However the results of a Salmonella infection experiment using mice suggested that wine was not effective e in preventing food-borne diseases in vivo.

L40 ANSWER 4 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2001:768601 The Genuine Article (R) Number: 474KC. Role of the outer membrane of Eschericia coli AG100 and Pseudomonas aeruginosa NCTC 6749 and resistance/susceptibility to monoterpenes of similar chemical structure. Griffin S G (Reprint); Wyllie S G; Markham J L. Univ Western Sydney, Ctr Biostruct & Biomol Res, Richmond, NSW 2753, Australia (Reprint). JOURNAL OF ESSENTIAL OIL RESEARCH (SEP-OCT 2001) Vol. 13, No. 5, pp. 380-386. ISSN: 1041-2905. Publisher: ALLURED PUBL CORP, 362 S SCHMALE RD, CAROL STREAM, IL 60188-2787 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Four pairs of oxygenated terpenes, with closely related chemical structures but considerably different minimum inhibitory concentration values (1) against F. aeruginosa or E. coh, showed differences in rate of cells **killed** over 2 h. Addition of polymyxin B nonapeptide (PMBN) as an outer membrane permeabilising agent was found to significantly increase the initial rates and overall numbers of cells **killed** for all compounds, The hydrocarbon limonene and the ester geranyl acetate, normally inactive, were also investigated. Both compounds had little killing effect when added alone to the cells but did show an enhanced killing capacity upon the addition of PBMN.

L40 ANSWER 5 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

1999:466466 Document No.: PREV199900466466. Survival of bacteria at a subfreezing temperature (-1degreeC). Tanaka, Yoshinori [Reprint author]; Ishino, Tsuyoshi [Reprint author]; Matsuba, Takashi [Reprint author]; Takayama, Hisao [Reprint author]; Ishida, Shigeru. Department of Bacteriology, Faculty of Medicine, Tottori University, Yonago, 683-0826, Japan. Yonago Acta Medica, (July, 1999) Vol. 42, No. 2, pp. 147-152. print.

CODEN: YOAMAQ. ISSN: 0513-5710. Language: English.

AB Preservation of foodstuffs at temperatures around -1degreeC has attracted special interest recently. We investigated whether bacteria contaminating foodstuffs, especially contaminating fish, were **killed** or survived at -1degreeC compared with 37degreeC. Survival rates of Escherichia coli K12 and Staphylococcus aureus IFO12732 in nutrient broth at -1degreeC for 7 days were 52% and 31%, respectively. However, the survival rate of Vibrio parahaemolyticus in nutrient broth containing 3% NaCl at -1degreeC for 7 days was only 0.03%. When the bacteria were kept in a soy sauce solution containing **alcohol** and some seasonings (the soy sauce solution) at -1degreeC, survival rates of **E. coli** K12 and S. aureus IFO12732 after 2 days were 56% and 54%, respectively, but V. parahaemolyticus was completely **killed** after 24 h at -1degreeC in the soy sauce solution. When **E. coli** K12 and O157 and V. parahaemolyticus were incubated at -1degreeC in the soy sauce solution containing some pieces of raw fish (the improved soy sauce solution), 3 strains of the bacteria were not **killed**. These results indicate that bacteria contaminating fish are not **killed** at -1degreeC and that storage of fish at -1degreeC is not always effective in diminishing food poisoning.

L40 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN

1998:642268 Document No. 129:274986 Reduction of Escherichia coli O157:H7 population in soy sauce, a fermented seasoning. Masuda, Susumu; Hara-Kudo, Yukiko; Kumagai, Susumu (Noda Inst. Sci. Res., Noda, 278,

Japan). Nippon Shoyu Kenkyusho Zasshi, 24(5), 275-281 (Japanese) 1998. CODEN: NSKZDP. ISSN: 0286-7958. Publisher: Nippon Shoyu Kenkyusho.

AB The pathogenic **E. coli**, **E. coli** O157:H7, was added to soy sauce, and the effect of soy sauce against the growth of O157:H7 was examined The incubation at 30°C in soy sauce diminished the viable cells for 9 days. The bactericidal effect of soy sauce against the O157 strain was dependent on the temperature condition. The bactericidal effect was weak at 18°C, and there was no cell reduction effect at below 4°C. Even at the low temps., the O157 did not propagate in the soy sauce. Soy sauce was bactericidal at high temps. and bacteriostatic at low temps. Those effect of soy sauce against O157:H7 was dependent on the NaCl concentration, **alc.** concentration, pH, the kinds of organic acids, addition of preservatives, temps., and treatment time. Soy sauce products confirmed to be safe from the contamination of **E. coli** O157:H7, because the bacterium will be **killed** by the soy sauce making processes such as fermentation, aging, and sterilization. As soy sauce products are preserved and sold at an ambient temperature, there is no chance for the bacterium to grow in the soy sauce products.

L40 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 2

91042421. PubMed ID: 2146486. **Alcohol** treatment of defective lambda lysogens is deletionogenic. Hayes S; Duncan D; Hayes C. (Department of Microbiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada.) Molecular & general genetics : MGG, (1990 Jun) Vol. 222, No. 1, pp. 17-24. Journal code: 0125036. ISSN: 0026-8925. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB We ascertained that transient exposure to ethanol, above 18%, was deletionogenic to an Escherichia coli strain with a fragment (12.5 kb) of bacteriophage lambda integrated within the chromosome. The lambda attL B.P' through P fragment provided a forward selection for mutants, and a target for mutagenesis. The cells were **killed** by thermal derepression of transcription and replication of the lambda fragment when transferred from 30 degrees to 42 degrees C. Survivor mutants, capable of forming colonies at 42 degrees C, were selected from untreated starting cells. About half no longer supported marker rescue of the lambda fragment imm lambda (immunity) region, comprising the cI repressor, and the PL and PR promoters. Ethanol treatment of starting cells increased the occurrence of imm lambda-defective clones to near 100%. The mutations responsible for the imm lambda defect were found to be large deletions (12 kb or more of DNA). Ethanol treatment of the starting cells also produced a 5- to 18-fold increase in the occurrence of **E. coli** pgl mutations, which likely arose by the deletion mechanism generating the imm lambda defects, since pgl was closely linked to the integrated lambda fragment. A unifying hypothesis for these observations was that ethanol was deletionogenic. The inclusion or substitution of the int-kil segment of the lambda fragment produced no real change in the spontaneous occurrence of large imm lambda deletions from the untreated cells. Substitution of this segment suppressed the deletionogenic effect of ethanol, implying a prerequisite for sequence homology or gene function from this interval.(ABSTRACT TRUNCATED AT 250 WORDS)

L40 ANSWER 8 OF 19 MEDLINE on STN DUPLICATE 3

88258341. PubMed ID: 3290376. Efficacy of various methods of sterilization of acupuncture needles. Sisco V; Winters L L; Zange L L; Brennan P C. (Department of Microbiology, National College of Chiropractic, Lombard, IL 60148.) Journal of manipulative and physiological therapeutics, (1988 Apr) Vol. 11, No. 2, pp. 94-7. Journal code: 7807107. ISSN: 0161-4754. Pub. country: United States. Language: English.

AB The iatrogenic transmission of hepatitis B virus by inadequately sterilized acupuncture needles recently has been reported. Because some

licensed chiropractors use acupuncture as a therapeutic modality, we have evaluated sterilization methods for these needles, which would be adaptable for use in a chiropractic office. Dry heat, boiling water, pressurized steam, sodium hypochlorite, and 70% **alcohol** were compared with a glass bead dry heat sterilizer originally developed for dental instruments. Presterilized acupuncture needles were contaminated with Bacillus stearothermophilus, Escherichia coli or Staphylococcus epidermidis and sterilized for intervals ranging from 5 sec to 30 min. The needles were then cultured to determine the efficacy of the sterilization regimen. Seventy percent **alcohol** was ineffective as a sterilization method. In terms of both time and convenience, the glass bead apparatus was the most efficient of the remaining methods tested. B. stearothermophilus-contaminated acupuncture needles were sterilized within 10 sec of exposure to preheated glass beads. Less than 10 sec exposure **killed E. coli** and S. epidermidis. A significant advantage of the glass bead sterilizer over the other methods was the absence of physical damage to the needles.

L40 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1987:418941 Document No. 107:18941 A molecular analysis of the RK mutatest. Gordon, Alasdair J. E.; Glickman, Barry W. (Dep. Biol., York Univ., Toronto, ON, M3J 1P3, Can.). Mutation Research, 190(4), 253-8 (English) 1987. CODEN: MUREAV. ISSN: 0027-5107.
AB The replicative killing (RK) test for detection of mutagens (in which Escherichia coli cells are **killed** by derepression of λ-DNA fragment based on temperature ≥39°) has been reported to show mutagenesis from EtOH; the RK tester strains CHY832 and SA431 of **E. coli** were hybridized with viral DNA. The expected extents of λ-DNA fragments in the strains were found but the complete mechanism of the RK mutatest is not understood.

L40 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1985:73951 Document No. 102:73951 **Alcohol**-induced suppression of the humoral immune response. Stolen, J. S.; Draxler, S.; Nagle, J. J. (Northeast Fish. Cent., Natl. Mar. Fish. Serv., Highlands, NJ, 07732, USA). Bulletin of Environmental Contamination and Toxicology, 34(1), 106-8 (English) 1985. CODEN: BECTA6. ISSN: 0007-4861.
AB Summer flounder (Paralichthys dentatus) pretreated with EtOH [64-17-5] or with EtOH + Aroclor 1254 [11097-69-1] showed a complete suppression of the immune response to formalin-**killed** human enteric Escherichia coli cells after their injection for 42 days. In nonpretreated fish agglutinating antibodies to **E. coli** were detected after 7 days. The EtOH + Aroclor 1254 pretreatment had a more pronounced immunosuppression that EtOH alone.

L40 ANSWER 11 OF 19 MEDLINE on STN DUPLICATE 4
84128718. PubMed ID: 6199044. Changes in streptonigrin lethality during adaptation of Escherichia coli to picolinic acid. Correlation with intracellular picolinate and iron uptake. Yeowell H N; White J R. Biochimica et biophysica acta, (1984 Mar 1) Vol. 797, No. 3, pp. 302-11. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB Uptake studies with [14C]picolinate and 55Fe3+ have provided an explanation for the change in streptonigrin killing on adaptation of Escherichia coli to picolinate, in terms of the available iron within the cell. When picolinic acid is added to a growing culture of **E. coli** an interval of bacteriostasis ensues; this adaptation period is followed by resumption of exponential growth. Addition of picolinate (4 mM) to a log phase culture of strain W3110 gave protection from the lethal action of streptonigrin (30 microM) when the two agents were added simultaneously. In contrast streptonigrin **killed** cells that had adapted to picolinate; however, a preincubation of adapted W3110 with

phenethyl **alcohol** protected the cells from streptonigrin lethality. [14C]Picolinate uptake studies showed that initially picolinate entered the cells, but that it was excluded from adapted cells; addition of phenethyl **alcohol** permitted the entry of picolinate into adapted W3110. The changes in streptonigrin killing parallel the changes in concentration of intracellular picolinate, which can chelate the iron required by streptonigrin for its bactericidal action. 55Fe3+ uptake studies showed that initially picolinate prevented iron accumulation by strain W3110, whereas adapted cells did take up iron in the presence of picolinate. Addition of phenethyl **alcohol** prevented any observed uptake of iron by adapted W3110. This modulation of iron transport by picolinate also affects streptonigrin lethality. Experiments with iron transport mutants showed that picolinate acted on both the enterochelin and citrate routes of uptake. Therefore picolinate affects the concentration of available iron within the cell both by (a) its intracellular presence resulting in chelation of iron and (b) its action on iron uptake; these effects explain the change in streptonigrin killing on adaptation of **E. coli** to picolinate.

L40 ANSWER 12 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

78282008 EMBASE Document No.: 1978282008. Possible sources of ethanol ante- and post-mortem: Its relationship to the biochemistry and microbiology of decomposition. Corry J.E.L.. Metrop. Police Forens. Sci. Lab., London, United Kingdom. Journal of Applied Bacteriology Vol. 44, No. 1, pp. 1-56 1978.

CODEN: JABAA4

Pub. Country: United Kingdom. Language: English.

AB Although ethanol can on rare occasions be detected in blood from living subjects who have not ingested **alcohol**, these levels never exceed 5 mg/100 ml. On the other hand, levels up to 150 mg/100 g have been detected in blood and tissues of putrefied human or rodent corpses. Ingestion of ethanol ante-mortem in these cases is known not to have taken place (in the case of the rodents), or is most unlikely to have taken place (in the case of humans). Production of ethanol has occurred, not only in tissues that have obviously putrefied, but within a relatively short time if temperatures are elevated (i.e. above about 15°C). Experience with decomposition of meat shows that high numbers of bacteria can be present without showing obvious signs of putrefaction. The limited evidence available suggests that ethanol is not formed post-mortem except by microbial action, and that ethanol is both produced and utilized, so that bodies with high initial levels will show a decrease, and bodies with low initial levels will show an increase. The method by which bacteria invade dead bodies is not entirely clear. However, the source appears to be mainly intestinal, although injury resulting in skin breakage immediately before death may introduce exogenous micro-organisms into the blood stream and throughout the body. There is evidence that bacteria may penetrate the intestinal walls during death and be distributed throughout the tissues in the blood stream, this may also occur during food absorption and from skin abrasions, etc. throughout life. Even after clinical death has occurred these organisms may be prevented from multiplying or actually **killed**, by the residual antimicrobial defences of the body, and the anaerobic organisms will be inhibited initially by the high Ph, but within a few hours, provided the temperature exceeds about 5°C, they will start to multiply. This primary invasion is probably reinforced by a secondary invasion of intestinal organisms, starting via the hepatic portal vein and the intestinal lymph system, and spreading round the body via the vascular system. Although the intestine harbours a wide variety of organisms, the majority obligate and fastidious anaerobes, only relatively few groups have been implicated as major colonizers of corpses during putrefaction; these include, in order of importance, Cl. perfringens (a vigorous saccharolytic, lipolytic

and proteolytic organism) and other Clostridium spp., enterobacteria (frequently, **E. coli** and Proteus spp.), Micrococcaeae (frequently Staph. aureus), streptococci and Bacillus spp. All of these are capable of producing ethanol from glucose and other substrates. In addition, a wider variety of organisms may be detected in the early stages of putrefaction, and these include yeasts, which may produce very high ethanol levels if present in sufficiently high numbers. Information on levels of substrates present shortly after death is sparse and further studies on this subject would be of interest. Glucose may be present in high levels in the liver and nearby blood and tissues, levels in the blood generally may be raised. Other possibly important sources are amino-acids (especially once proteolysis has commenced), glycerol (formed during fat hydrolysis), and lactate which occurs widely and at levels over 100 mg/100 g in all tissues. There is evidence that all these compounds can serve as substrates for ethanol production by bacteria commonly found in corpses. Forensic scientists must, therefore, always bear in mind that specimens of human tissue containing micro-organisms, particularly specimens taken from corpses, may contain ethanol produced by microbial fermentation, and that extreme caution should be exercised when assessing the significance of post-mortem ethanol.

L40 ANSWER 13 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

78161559 EMBASE Document No.: 1978161559. Enhanced susceptibility of mice to combinations of Δ9 tetrahydrocannabinol and live or **killed** gram negative bacteria. Bradley S.G.; Munson A.E.; Dewey W.L.; Harris L.S.. Dept. Microbiol., Med. Coll. Virginia, Virginia Commonwlth. Univ., Richmond, Va. 23298, United States. Infection and Immunity Vol. 17, No. 2, pp. 325-329 1977.
CODEN: INFIBR
Pub. Country: United States. Language: English.

AB Combinations of Δ9-tetrahydrocannabinol (Δ9-THC) and bacterial endotoxin were shown to be hyperadditively toxic for mice. A variety of purified lipopolysaccharide (LPS) preparations elicited enhanced mortality in combination with Δ9-THC. Escherichia coli O26:B6 LPS (Boivin preparation) at an essentially nonlethal dose of 2.5 mg/kg reduced the dose of Δ9-THC required to kill 50% of the treated mice from ca. 350 to 150 mg/kg. Inbred BALB, DBA, and C3H/HeCr mice, noninbred ICR mice, and hybrid CDF1 and BDF1 mice were hyperactive to combinations of Δ9-THC and LPS. Moreover, a variety of heat- **killed** intestinal and gram-negative bacteria, live **E. coli**, and complexes of lipid A with a variety of proteins substituted for LPS in the synergistic toxicity of LPS and Δ9-THC. Extracts of marijuana also elicited hyperreactivity to LPS. The hyperadditive lethality of combinations of Δ9-THC and LPS was markedly less in mice rendered refractory to LPS or Δ9-THC by repeated administration of LPS or Δ9-THC, respectively.

L40 ANSWER 14 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

74186265 EMBASE Document No.: 1974186265. The photosensitizing action of carcinogens. I. The action of 2 naphthylamine on Escherichia coli K 12 and Paramecium caudatum. Ellis S.P.; Smith R.C.; Neely W.C.. Dept. Chem., Auburn Univ., Auburn, Ala. 36830, United States. Canadian Journal of Microbiology Vol. 20, No. 2, pp. 125-129 1974.
CODEN: CJMIAZ
Language: English.

AB Cultures of Paramecium caudatum incubated with 7 x 10-7 M 2 naphthylamine were rapidly **killed** when exposed to light of 366 nm. Cultures not exposed to the amine were unaffected by the light; cultures kept in the dark were unaffected by the amine. Escherichia coli K 12 populations were markedly reduced after irradiation of suspensions in water containing

3 x 10-4 M 2 naphthylamine with light simulating natural sunlight in intensity and wavelength distribution. Suspensions of **E. coli** in deionized water were unaffected by the light and **E. coli** suspended in solutions of the amine but kept in the dark were also unaffected. Since 2 naphthylamine is a known water pollutant, these results may be of ecological importance.

L40 ANSWER 15 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

75004075 EMBASE Document No.: 1975004075. The immunologic role of the ethanol soluble enterobacterial common antigen versus experimental renal infection. McLaughlin J.C.; Domingue G.J.. Dept. Surg., Sect. Urol., Tulane Univ. Sch. !ed., New Orleans, La. 70112, United States. IMMUNOL.COMMUN. Vol. 3, No. 1, pp. 51-75 1974.
CODEN: XXXXXB
Language: English.

AB Members of the Enterobacteriaceae contain a common antigen (CA) which is found in the ethanol soluble fraction (ESF) of heat **killed** culture supernates. The ESF of an **E. coli** 06 strain was shown to be virtually endotoxin free. Preliminary chemical studies revealed that the dry ESF, including salts, contained 20% protein and less than 1% carbohydrate. Chloroform/methanol soluble lipid accounted for approximately 2% of the material. Vaccination of rabbits with such enterobacterial CA elicited protection against renal disease due to retrograde challenge with Proteus mirabilis or to hematogenous challenge with **E. coli** 075. Protection was not demonstrated against a heavily encapsulated strain of Klebsiella pneumoniae which, as demonstrated by in vitro phagocytosis, was not opsonized by antibody to CA. These results suggest that further investigation of enterobacterial CA as a vaccine is warranted.

L40 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN

1967:516075 Document No. 67:116075 Disinfecting action of 4-(p-dialkylaminophenyl)pyridine derivatives. Kolomoitsev, L. R.; Alfer'eva, M. A.; Matvienko, N. I.; Sheinkman, A. K. (Donets'k. Med. Inst., Donetsk, USSR). Mikrobiologichnii Zhurnal (1934-1977), 29(4), 342-4 (Ukrainian) 1967. CODEN: MZUKAV. ISSN: 0026-3664.

AB The antibacterial properties of 4-(p-dibutylaminophenyl)pyridine-HCl (I), 4-(p-dimethylaminophenyl)pyridine-MeI (II), and N-(β-hydroxyethyl)-4-(p-dimethylaminophenyl)pyridinium chloride (III) were tested against Escherichia coli, Staphylococcus aureus, Bacillus anthracoides, and Bact. proteus vulgaris [Proteus vulgaris]. The prepns. are yellow crystalline powders, slightly soluble in water, but readily soluble in **alc**. The solns. of the prepns. do not decompose when heated to 100°, and do not lose their bactericidal properties. They are only slightly toxic. The prepns. were used in concns. of 2, 1, 0.5, and 0.25% in water. Test objects saturated with the bacterial suspensions were seeded on nutritive media containing the prepns. studied and observed for 24 hrs. I in 0.25% concentration sterilized the test objects infected with **E. coli**, S. aureus, and B. anthracoides within 15 min.; Proteus vulgaris were more resistant and were **killed** within about 6 hrs. All of the microorganisms were resistant to II and III, and were **killed** only after a longer period of time.

L40 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN

1957:48410 Document No. 51:48410 Original Reference No. 51:8989a-c Changes caused by injurious agents in the permeability of surviving cells of liver and kidney. Opie, Eugene L. (Rockefeller Inst. Med. Research, New York, NY). Journal of Experimental Medicine, 104, 897-919 (Unavailable) 1956. CODEN: JEMEAV. ISSN: 0022-1007.

AB cf. C.A. 50, 15808c. Slices of liver and kidney, immersed in oxygenated buffered Krebs-Ringer solution at 38°, were exposed to a variety of

chemical and phys. agents and their permeability (I) measured. I was increased under conditions in which N replaced O, the temperature was raised to 58°, EtOH was added to make a dilution of 1/100-1/5000 (but not 1/20,000), the mol. concentration was raised 2-fold by NaCl, and when one of the following was added: CHCl3, a filtrate of Escherichia, coli in a final dilution of 1/10,000, somatic antigen of Shigella paradysenteriae in a dilution of 1/1000-1/100,000, a suspension of **killed** typhoid bacilli of 1/1000-1/100,000 dilution, and urea in concns. of 0.01-0.1M. I was not increased by the addition of glucose, histamine, diphtheria toxin, or an **E. coli** filtrate of 1/10,000 dilution in the presence of a 2-fold increase in mol. concentration

L40 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1956:49234 Document No. 50:49234 Original Reference No. 50:9507d-h A comparative biochemical and immunological study of the directed mutability in some bacteria from the intestines. Belozerskii, A. N.; Spirin, A. S.; Kudlai, D. G.; Skavronskaya, A. G. (Moscow State Univ.). Biokhimiya (Moscow), 20, 686-95 (Unavailable) 1955. CODEN: BIOHAO. ISSN: 0320-9725.
AB Studies were conducted with (1) Escherichia coli, strain CM, grown in glucose-free Tyrode medium in the presence of heat-**killed** Salmonella paratyphi; (2) S. breslau Number 70; (3) Alkaligenes 11-IV-4 which was evolved from **E. coli** CM by culturing the latter in the presence of heat-**killed** S. Breslau Number 70; and (4) S. paratyphi mutant 12-IV-4 evolved from culturing Alkaligenes 11-IV-4 on synthetic medium in the presence of heat-**killed** S. breslau Number 70. All 4 types of bacteria were grown in parallel series on portions of the same batch of the same type of culture medium at 37° for 20 hrs. Growth was washed off with saline, washed again with saline, **alcohol** and ether, and vacuum dried. The chemical characteristics of the corresponding bacterial masses were established by analyzing them for total N, total P, for purine base N, pentoses, reducing substances (after 4 hrs. hydrolysis with 1 N HCl), for total nucleic acids, deoxyribonucleic acid, ribonucleic acid, protein and polysaccharides. **E. coli** grown on synthetic medium in the presence of heat-**killed** S. breslau Number 70 undergoes basic mutation changes which are reflected in its chemical composition and immunological (antigenic) properties. Alkaligenes evolved from **E. coli** acquires a chemical and immunological entity all its own. The new strain which is evolved from the newly developed Alkaligenes strain, when again grown in the presence of heat-**killed** S. breslau Number 70 is a paratyphoid type of mutant, the chemical and immunologic characteristics of which are partly those of the original S. breslau and partly those of the Alkaligenes. The chemo-immunological analysis of the fractions indicated a phylogenetic connection between the experimentally evolved types and the original cultures. The nature of the chemical and immunological changes which had taken place present evidence of the complex structure of the protoplasm of the intestinal microorganisms under study. Among the protein complexes of the bacterial cells are found two specifically distinct components, one labile which changes from one biological form to another within narrow specific ramifications and a protoplasmic component more stable and equally specific within the ramifications of a broader systematic group.

L40 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1954:18847 Document No. 48:18847 Original Reference No. 48:3458d-f The use of metabolites in the restoration of the viability of heat and chemically inactivated Escherichia coli. Heinmets, F.; Taylor, W. W.; Lehman, J. J. (Naval Med. Research Lab., Camp Lejeune, NC). Journal of Bacteriology, 67, 5-12 (Unavailable) 1954. CODEN: JOBAAY. ISSN: 0021-9193.
AB Suspensions of **E. coli**, strain B/r, which had apparently been **killed** by the action of heat, Cl2, zephiran

chloride, **alc.**, or H2O2, were found to contain viable cells when incubated with various metabolites of the tricarboxylic series. When the apparently sterile suspensions were incubated in buffer or in nutrient broth, no viable cells could be demonstrated. The following metabolites were the most effective in producing reactivation: (1) with heat-"**killed**" cells, Na citrate, lactic acid, and oxalacetic acid; (2) Cl2-"**killed**" cells, Na citrate, malic acid, and oxalacetic acid; (3) H2O2-"**killed**" cells, Na citrate, lactic acid, and cis-aconitic acid; (4) zephiran chloride-"**killed**" cells, Na citrate, lactic acid, cis-aconitic acid, and isocitric acid; (5) **alc.**-"**killed**" cells, cis-aconitic acid, α-ketoglutaric acid, and succinic acid. The combination of 11 metabolites produced the highest reactivation. It is probable that such reactivation is concerned with resynthesis of enzymes and re-establishment of cyclic processes. Conventional testing and culturing are not adequate to determine complete sterility.

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=> s allergen expressing bacteria
L41          0 ALLERGEN EXPRESSING BACTERIA

=> s allergen expressing E coli
L42          0 ALLERGEN EXPRESSING E COLI

=> s caplan m?/au
L43       1416 CAPLAN M?/AU

=> s l43 and microbial delivery
L44          1 L43 AND MICROBIAL DELIVERY

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L44  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
AN   2001:676622  CAPLUS
DN   135:225857
TI   Microbial delivery system
IN   Caplan, Michael
PA   Panacea Pharmaceuticals, LLC, USA
SO   PCT Int. Appl., 57 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 14
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
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PI   WO 2001066136        A2    20010913    WO 2000-US33121        20001206
     WO 2001066136        A3    20011227
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             HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
             LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
             SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU,
             ZA, ZW
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
             BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     CA 2403292           A1    20010913    CA 2000-2403292        20001206
     AU 2001019510        A5    20010917    AU 2001-19510          20001206
     EP 1272213           A2    20030108    EP 2000-982485         20001206
     EP 1272213           B1    20060308
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
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	JP 2004527450	T	20040909	JP 2001-564788	20001206
	PT 1272213	T	20060630	PT 2000-982485	20001206
	AU 765211	B2	20030911	AU 2001-43769	20010508
PRAI	US 2000-195035P	P	20000406		
	AU 1996-72433	A3	19960923		
	US 2000-731375	A	20001206		
	WO 2000-US33121	W	20001206		

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L46 3 DUP REMOVE L45 (7 DUPLICATES REMOVED)

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L46 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2005:259357 Document No. 142:334946 Recombinant allergens with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex allergens. **Caplan, Michael J.**; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 2005063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.

AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein allergen. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein allergen. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as **E. coli** . Any allergen may be used in the inventive methods. Particularly preferred allergens are anaphylactic allergens including protein allergens found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L46 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
1999394992. PubMed ID: 10464133. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. **Caplan M S**; Miller-Catchpole R; Kaup S; Russell T; Lickerman M; Amer M; Xiao Y; Thomson R Jr. (Department of Pediatrics, Northwestern University Medical School, Evanston Hospital, Evanston, Illinois, USA.) Gastroenterology, (1999 Sep) Vol. 117, No. 3, pp. 577-83. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: Neonatal necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease of premature infants partly caused by intestinal bacterial proliferation. Because bifidobacteria are thought to reduce the risk for intestinal disturbances associated with pathogenic bacterial colonization, we hypothesized that exogenous bifidobacterial supplementation to newborn rats would result in intestinal colonization and a reduction in the incidence of neonatal NEC. METHODS: Newborn rat pups were given Bifidobacterium infantis (10(9) organisms per animal daily), Escherichia coli, or saline control and exposed to the NEC protocol consisting of formula feeding (Esbilac; 200 cal. kg(-1). day(-1)) and asphyxia (100% N(2) for 50 seconds followed by cold exposure for 10 minutes). Outcome measures included stool and intestinal microbiological evaluation, gross and histological evidence of NEC, plasma endotoxin concentration, intestinal phospholipase A(2) expression, and estimation of

intestinal mucosal permeability. RESULTS: Bifidobacterial supplementation resulted in intestinal colonization by 24 hours and appearance in stool samples by 48 hours. Bifidobacteria-supplemented animals had a significant reduction in the incidence of NEC compared with controls and **E. coli**-treated animals (NEC, 7/24 B. infantis vs. 19/27 control vs. 16/23 **E. coli**; P < 0.01). Plasma endotoxin and intestinal phospholipase A(2) expression were lower in bifidobacteria-treated pups than in controls, supporting the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC. CONCLUSIONS: Intestinal bifidobacterial colonization reduces the risk of NEC in newborn rats.

L46 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 2
95073279. PubMed ID: 7982271. Altered mitochondrial redox responses in gram negative septic shock in primates. Simonson S G; Welty-Wolf K; Huang Y T; Griebel J A; **Caplan M S**; Fracica P J; Piantadosi C A. (Department of Medicine, Duke University Medical Center, Durham, NC 27710.) Circulatory shock, (1994 May) Vol. 43, No. 1, pp. 34-43. Journal code: 0414112. ISSN: 0092-6213. Pub. country: United States. Language: English.

AB Gram negative sepsis causes changes in oxygen supply-demand relationships. We have used a primate model of hyperdynamic gram negative sepsis produced by intravenous infusion of Escherichia coli (**E. coli**) to evaluate sepsis-induced alterations in mitochondrial oxidation-reduction (redox) state in muscle in vivo. The redox state of cytochrome a,a3, the terminal member of the intramitochondrial respiratory chain, was assessed in the intact forearm by near-infrared (NIR) spectroscopy. The muscle NIR data were compared to routine measures of oxygen delivery (DO2) and oxygen consumption (VO2). After **E. coli** infusion and fluid resuscitation, DO2 and VO2 showed minimal changes through 24 hr of sepsis. In contrast, changes in cytochrome a,a3 redox state evaluated by NIR occurred within a few hours and were progressive. Mitochondrial functional responses were correlated with structural changes observed on serial muscle biopsies. Gross morphological changes in muscle mitochondria were present in some animals as early as 12 hr, and, in most animals, by 24 hr. The morphologic changes were consistent with decreases in oxidative capacity as suggested by NIR spectroscopy. The NIR data also suggest that two mechanisms are operating to explain abnormalities in oxygen metabolism and mitochondrial function in lethal sepsis. These mechanisms include an early defect in oxygen provision to mitochondria that is followed by a progressive loss in functional cytochrome a,a3 in the muscle.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	387.36	387.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-24.96	-24.96

STN INTERNATIONAL LOGOFF AT 12:29:34 ON 09 JAN 2007

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Welcome to STN International! Enter x:X

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

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NEWS 2	OCT 02	CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt
NEWS 3	OCT 19	BEILSTEIN updated with new compounds
NEWS 4	NOV 15	Derwent Indian patent publication number format enhanced
NEWS 5	NOV 19	WPIX enhanced with XML display format
NEWS 6	NOV 30	ICSD reloaded with enhancements
NEWS 7	DEC 04	LINPADOCDB now available on STN
NEWS 8	DEC 14	BEILSTEIN pricing structure to change
NEWS 9	DEC 17	USPATOLD added to additional database clusters
NEWS 10	DEC 17	IMSDRUGCONF removed from database clusters and STN
NEWS 11	DEC 17	DGENE now includes more than 10 million sequences
NEWS 12	DEC 17	TOXCENTER enhanced with 2008 MeSH vocabulary in MEDLINE segment
NEWS 13	DEC 17	MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary
NEWS 14	DEC 17	CA/CAplus enhanced with new custom IPC display formats
NEWS 15	DEC 17	STN Viewer enhanced with full-text patent content from USPATOLD
NEWS 16	JAN 02	STN pricing information for 2008 now available
NEWS 17	JAN 16	CAS patent coverage enhanced to include exemplified prophetic substances
NEWS 18	JAN 28	USPATFULL, USPAT2, and USPATOLD enhanced with new custom IPC display formats
NEWS 19	JAN 28	MARPAT searching enhanced
NEWS 20	JAN 28	USGENE now provides USPTO sequence data within 3 days of publication
NEWS 21	JAN 28	TOXCENTER enhanced with reloaded MEDLINE segment
NEWS 22	JAN 28	MEDLINE and LMEDLINE reloaded with enhancements
NEWS 23	FEB 08	STN Express, Version 8.3, now available
NEWS 24	FEB 20	PCI now available as a replacement to DPCI
NEWS 25	FEB 25	IFIREF reloaded with enhancements
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NEWS 27	FEB 29	WPINDEX/WPIDS/WPIX enhanced with ECLA and current U.S. National Patent Classification
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L6 ANSWER 1 OF 12 MEDLINE on STN
95046935. PubMed ID: 7958476. Mucosal vaccines based on the use of cholera toxin B subunit as immunogen and **antigen carrier**.
Lebens M; Holmgren J. (Department of Medical Microbiology and Immunology, University of Goteborg, Sweden.) Developments in biological standardization, **(1994)** Vol. 82, pp. 215-27. Ref: 39. Journal code: 0427140. ISSN: 0301-5149. Pub. country: Switzerland. Language: English.
AB Stimulation of strong mucosal IgA immune responses as a basis for vaccine-induced protection against various pathogens has proved difficult. Most soluble protein antigens administered either parenterally or oral-mucosally have given disappointing results. A notable exception in this regard are cholera toxin (CT) and, particularly in humans, its non-toxic B subunit pentamer moiety (CTB) both of which stimulate a strong intestinal IgA antibody response and long-lasting immunological memory. Based on this, CTB has become an important component in recently developed oral vaccines against cholera and diarrhea caused by enterotoxigenic **E. coli**. The strong immunogenicity of CT and CTB can to a large extent be explained by their ability to bind to receptors on the intestinal mucosal surface. This has promoted much recent interest in the use of CTB as an oral delivery carrier for other vaccine-relevant antigens. Oral administration of antigens coupled to CTB either chemically or genetically has in several systems been found to markedly potentiate both intestinal and extra-intestinal IgA immune responses against the CTB-coupled antigens and to elicit substantial circulating antibody responses. In contrast to CTB, CT also has strong adjuvant properties for stimulating mucosal IgA immune responses to unrelated, non-coupled antigens after oral co-immunization. This adjuvant activity appears to be closely linked to the A subunit-catalyzed ADP-ribosylating action of CT leading to enhanced cyclic AMP formation in the affected cells.

L6 ANSWER 2 OF 12 MEDLINE on STN
93254204. PubMed ID: 1302285. Extracellular export of Shiga toxin B-subunit/haemolysin A (C-terminus) fusion protein expressed in Salmonella typhimurium aroA-mutant and stimulation of B-subunit specific antibody responses in mice. Su G F; Brahmbhatt H N; de Lorenzo V; Wehland J; Timmis K N. (Department of Microbiology, GBF-National Research Centre for Biotechnology, Braunschweig, Germany.) Microbial pathogenesis, **(1992 Dec)** Vol. 13, No. 6, pp. 465-76. Journal code: 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The Shiga toxin B-subunit has been fused to the 23-kD C-terminus of Escherichia coli haemolysin A (HlyA) and exported from attenuated **antigen carrier** strain of Salmonella typhimurium aroA (SL3261). The expression of the gene fusion under the control of a synthetic modified beta-lactamase promoter (constitutive expression) and under the iron-regulated aerobactin promoter showed that the fusion protein could be stably expressed and exported out of the bacterial cell in significant amounts so long as high copy number plasmids were not used. Oral and i.p. immunization of mice with the hybrid salmonellae resulted in significant B-subunit specific mucosal and serum antibody responses. A comparative analysis of the location of hybrid proteins in the **antigen carrier** bacterial cell (i.e. cytoplasmic

expression and extracellular export) has shown that both modes of expression result in antigen-specific immune responses. This is the first report demonstrating that foreign polypeptides fused to the 23-kD C-terminus of **E. coli** haemolysin A can be exported from attenuated Salmonella vaccine strains and that such exported polypeptides can result in antigen-specific immune responses.

L6 ANSWER 3 OF 12 MEDLINE on STN

91100317. PubMed ID: 1987133. Expression of the cloned Escherichia coli O9 rfb gene in various mutant strains of Salmonella typhimurium. Sugiyama T; Kido N; Komatsu T; Ohta M; Kato N. (Department of Bacteriology, Nagoya University School of Medicine, Aichi, Japan.) Journal of bacteriology, **(1991 Jan)** Vol. 173, No. 1, pp. 55-8. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB To investigate the effect of chromosomal mutation on the synthesis of rfe-dependent Escherichia coli O9 lipopolysaccharide (LPS), the cloned **E. coli** O9 rfb gene was introduced into Salmonella typhimurium strains defective in various genes involved in the synthesis of LPS. When **E. coli** O9 rfb was introduced into S. typhimurium strains possessing defects in rfb or rfc, they synthesized **E. coli** O9 LPS on their cell surfaces. The rfe-defective mutant of S. typhimurium synthesized only very small amounts of **E. coli** O9 LPS after the introduction of **E. coli** O9 rfb. These results confirmed the widely accepted idea that the biosynthesis of **E. coli** O9-specific polysaccharide does not require rfc but requires rfe. By using an rfbT mutant of the **E. coli** O9 rfb gene, the mechanism of transfer of the synthesized **E. coli** O9-specific polysaccharide from **antigen carrier** lipid to the R-core of S. typhimurium was investigated. The rfbT mutant of the **E. coli** O9 rfb gene failed to direct the synthesis of **E. coli** O9 LPS in the rfc mutant strain of S. typhimurium, in which rfaL and rfbT functions are intact, but directed the synthesis of the precursor. Because the intact **E. coli** O9 rfb gene directed the synthesis of **E. coli** O9 LPS in the same strain, it was suggested that the rfaL product of S. typhimurium and rfbT product of **E. coli** O9 cooperate to synthesize **E. coli** O9 LPS in S. typhimurium.

L6 ANSWER 4 OF 12 MEDLINE on STN

90198517. PubMed ID: 2576522. Oral vaccination of rats with live avirulent Salmonella derivatives expressing adhesive fimbrial antigens of uropathogenic Escherichia coli. Schmidt G; Hacker J; Wood G; Marre R. (Forschungsinstitut Borstel, F.R.G.) FEMS microbiology immunology, **(1989 Mar)** Vol. 1, No. 4, pp. 229-35. Journal code: 8901230. ISSN: 0920-8534. Pub. country: Netherlands. Language: English.

AB The avirulent Salmonella typhimurium F885 was transformed with a plasmid carrying the cloned S fimbriae genes of a uropathogenic Escherichia coli. The resulting transformant (F885-1) produced efficiently **E. coli** S fimbriae and was used for live oral vaccination of rats. For comparison rats were immunized subcutaneously with isolated S fimbriae. Both routes of vaccination resulted in a significant IgG antibody response to S fimbriae. In addition live oral vaccination induced a serum IgA response against S fimbriae. After transurethral infection of rats with a S fimbriae producing **E. coli** a 10-fold reduction of bacterial counts in the kidney was observed in rats orally vaccinated with F885-1 as compared to unvaccinated controls. This study suggests that the avirulent Salmonella F885 may be used as a fimbrial **antigen carrier** for oral vaccination against renal infections.

L6 ANSWER 5 OF 12 MEDLINE on STN

76033569. PubMed ID: 1101370. Antibody production by human colostral cells. I. Immunoglobulin class, specificity, and quantity. Ahlstedt S; Carlsson B; Hanson L A; Goldblum R M. Scandinavian journal of immunology, **(1975 Sep)** Vol. 4, No. 5-6, pp. 535-9. Journal code: 0323767. ISSN: 0300-9475. Pub. country: Norway. Language: English.

AB The production of antibody by human colostral cells was assayed by the hemolysis in-gel technique. When sheep erythrocytes coated with O antigens from frequently encountered Escherichia coli bacteria were used as detector cells and anti-IgA serum was added for development, numerous plaque-forming cells (PFC) were demonstrated in all samples tested. In contrast, plaques were rarely seen in the presence of anti-IgG developing serum. The direct (IgM) plaques occasionally noted with both antigen-coated and uncoated sheep erythrocytes were mainly due to the production of heterophil antibodies, since they were not formed when human erythrocytes were used as O-**antigen carriers**. A strikingly high number of the colostral lymphocytes formed antibodies to the **E. coli** antigens, up to 8%. This suggests that these cells represent a rather selective population--possibly cells from the gastrointestinal tract exposed to enteric bacteria. The large number of plaques observed, the predominance of the cells forming IgA antibodies, and the marked changes in PFC number in relationship to parturition pose a number of questions relevant to the antibody-producing colostrum cells and their relationship to the secretory immune system.

L6 ANSWER 6 OF 12 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

1989062675 EMBASE Oral vaccination of rats with live avirulent Salmonella derivatives expressing adhesive fimbrial antigens of uropathogenic Escherichia coli.
Schmidt, G.; Hacker, J.; Wood, G.; Marre, R.. Forschungsinstitut Borstel, 2061 Borstel, Germany.
FEMS Microbiology Immunology Vol. 47, No. 4, pp. 229-235 **1989.**
ISSN: 0920-8534. CODEN: FMIMEH
Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 911212. Last Updated on STN: 911212

AB The avirulent Salmonella typhimurium F885 was transformed with a plasmid carrying the cloned S fimbriae genes of a uropathogenic Escherichia coli. The resulting transformant (F885-1) produced efficiently **E. coli** S fimbriae and was used for live oral vaccination of rats. For comparison rats were immunized subcutaneously with isolated S fimbriae. Both routes of vaccination resulted in a significant IgG antibody response to S fimbriae. In addition live oral vaccination induced a serum IgA response against S fimbriae. After transurethral infection of rats with a S fimbriae producing **E. coli** a 10-fold reduction of bacterial counts in the kidney was observed in rats orally vaccinated with F885-1 as compared to unvaccinated controls. This study suggests that the avirulent Salmonella F885 may be used as a fimbrial **antigen carrier** for oral vaccination against renal infections.

L6 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN

1999:761959 Document No. 132:45567 Expression of CS3 from enterotoxigenic Escherichia coli in Shigella flexneri 2a and immunogenicity of the recombinant strain. Han, Zhaozhong; Ying, Tianyi; Cao, Yong; Rui, Xianliang; Zhang, Zhaoshan; Su, Guofu; Huang, Cuifen (Beijing Institute of Biotechnology, Beijing, 100071, Peop. Rep. China). Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao, 15(5), 719-723 (Chinese) **1999.**
CODEN: ZSHXF2. ISSN: 1007-7626. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui.

AB A host-plasmid balancing system was established based on asd gene in a candidate vaccine strain(T32) of Shigella flexneri 2a. Asd gene of T32 was amplified by polymerase chain reaction(PCR), and its structural gene

fragment was replaced by human interleukin 2 gene. The mutated asd gene was introduced to T32 genome by homol. recombination. The resulted bacteria strain (FaD) was used as **antigen carrier** to express Escherichia coli surface antigen CS3 of enterotoxigenic **E. coli**, which was expressed on a complementary plasmid carrying asd gene from Streptococcus mutans. The plasmid could stably be maintained and expressed CS3 in the host cell without any antibiotic selection. Antibodies against CS3 could be detected in sera of mice immunized with recombinant bacteria either orally or s.c., and mice immunized by either route could be protected from challenging with virulent strain of the same serotype. All results indicate that the recombinant constructed can be used as bi-valent vaccine candidate for prevention of bacterial diarrhea.

L6 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN
1997:719672 Document No. 128:21852 Immunogenic carrier system against gonadotropin releasing hormone (GnRH). Van Der Zee, Anna; Van Die, Irma Marianne; Hoekstra, Willem Pieter Martin; Gielen, Josephus Theodorus (Akzo Nobel N.V., Neth.). U.S. US 5684145 A **19971104**, 28 pp., Division of U.S. Ser. No. 78,661, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1995-453588 19950530. PRIORITY: NL 1992-1775 19920618; US 1993-78661 19930616.
AB The present invention is concerned with vaccination of mammals against GnRH. The vaccine comprises a GnRH peptide conjugate to **E. coli** fimbrial-filaments and elicits an immune response against GnRH.

L6 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN
1996:123718 Document No. 124:173426 Peptides used as carriers in immunogenic constructs suitable for development of synthetic vaccines. Cohen, Irun R.; Fridkin, Matityahu; Konen-Waisman, Stephanie (Yeda Research and Development Co., Ltd., Israel). PCT Int. Appl. WO 9531994 A1 **19951130**, 32 pp. DESIGNATED STATES: W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US6575 19950524. PRIORITY: IL 1994-109790 19940525.
AB The invention relates to conjugates of poorly immunogenic antigens, e.g., peptides, proteins and polysaccharides, with a synthetic peptide carrier constituting a T cell epitope derived from the sequence of **E. coli** hsp65 (GroEL), or an analog thereof, said peptide or analog being capable of increasing substantially the immunogenicity of the poorly immunogenic antigen. A suitable peptide according to the invention is Pep287e, which corresponds to positions 437-453 of the **E. coli** hsp65 mol. In example, Pep287e was synthesized and conjugated with Citrobacter freundii-derived/protein and nucleic acid and polysaccharide-containing Vi fragment for use as immunogen to stimulate lymph node proliferation.

L6 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN
1992:468120 Document No. 117:68120 Safety, immunogenicity, and efficacy in monkeys and humans of invasive Escherichia coli K-12 hybrid vaccine candidates expressing Shigella flexneri 2a somatic antigen. Kotloff, Karen L.; Herrington, Deirdre A.; Hale, Thomas L.; Newland, John W.; Van de Verg, Lillian; Cogan, John P.; Snoy, Phillip J.; Sadoff, Jerald C.; Formal, Samuel B.; Levine, Myron M. (Sch. Med., Univ. Maryland, Baltimore, MD, 21201, USA). Infection and Immunity, 60(6), 2218-24 (English) **1992**. CODEN: INFIBR. ISSN: 0019-9567.
AB A live, oral Shigella vaccine, constructed by transfer of the 140-MDa invasiveness plasmid from S. flexneri 5 and the chromosomal genes encoding

the group- and type-specific O antigen of S. flexneri 2a to **E. coli** K-12, was tested in humans. Designated EcSf2a-1, this vaccine produced adverse reactions (fever, diarrhea, or dysentery) in 4 (31%) of 13 subjects who ingested a single dose of 1.0 + 109 CFU, while at better-tolerated doses (5.0 + 106 to 5.0 + 107 CFU), it provided no protection against challenge with S. flexneri 2a. A further-attenuated aroD mutant derivative, EcSf2a-2, was then tested. Rhesus monkeys that received EcSf2a-2 in 3 oral doses of 1.5 + 1011 CFU experienced no increase in gastrointestinal symptoms compared with a control group that received an **E. coli** K-12 placebo. Compared with controls, the vaccinated monkeys were protected against shigellosis after challenge with S. flexneri 2a (60% efficacy). In humans, EcSf2a-2 was well tolerated at inocula ranging from 5.0 + 106 to 2.1 + 109 CFU. However, after a single dose of 2.5 + 109 CFU, 4 (17%) of 23 subjects experienced adverse reactions, including fever (3 subjects) and diarrhea (1 subject), and after a single dose of 1.8 + 1010 CFU, 2 of 4 subjects developed dysentery. Recipients of 3 doses of 1.2-2.5 + 109 CFU had significant rises in serum antibody to lipopolysaccharide (61%) and invasiveness plasmid antigens (44%) and in gut-derived IgA antibody-secreting cells specific for lipopolysaccharide (100%) and invasiveness plasmid antigens (60%). Despite its immunogenicity, the vaccine conferred only 36% protection against illness (fever, diarrhea, or dysentery) induced by exptl. challenge. These findings illustrate the use of an epithelial cell-invasive **E. coli** strain as a carrier for Shigella antigens.

L6 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN

1992:405863 Document No. 117:5863 Regulation of the immune response to hepatitis B virus and human serum albumin. III. Induction of anti-albumin antibody secretion in vitro by C-gene-derived proteins in peripheral B cells from chronic carriers of HBsAg. Hellstroem, U. B.; Sylvan, S. P. E. (Dep. Infect. Dis., Karolinska Inst., Stockholm, S-11489, Swed.). Scandinavian Journal of Immunology, 35(1), 53-62 (English) **1992.** CODEN: SJIMAX. ISSN: 0300-9475.

AB The circulatory pool of B cells from the majority (11/13) of chronic hepatitis B surface antigen (HBsAg) carriers contained sensitized B cells with the capacity to secrete IgG antibodies with specificity for human serum albumin (HSA), when stimulated with **E. coli** -derived core protein at low concns. in vitro. The IgG anti-HSA secretion was dependent upon and regulated by T cells, and optimal secretion was obtained at T/B-cell ratios of 1.0-4.0, varying for different individuals. The level of anti-HSB secretion was higher for patients with on-going viral replication as assessed by hepatitis B virus (HBV)-DNA in serum. Culture supernatants containing anti-HSA antibodies also contained anti-HBc antibodies, as detected by ELISA where the solid phase was charged with **E. coli**-derived core protein, or the synthetic peptides corresponding to the 75-84 and 132-147 sequences in the C region of HBV. In contrast, IgG anti-HBc (**E. coli**-derived), but not anti-HSA or anti-HBc 75-84,132 147 antibodies, were detected at similar T/B-cell ratios in cell cultures from 5/6 individuals with naturally acquired immunity to hepatitis B. Thus, peripheral B cells from the majority of HB-immune donors are sensitized to unique (e.g. non-albumin associated) structures in the nucleocapsid of HBV, while B cells in the majority of chronic HBsAg carriers are sensitized to linear C-gene-derived structures in association with the host self-component HSA.

L6 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2008 ACS on STN

1990:476015 Document No. 113:76015 Outer membrane PhoE protein of Escherichia coli as a carrier for foreign antigenic determinants: immunogenicity of epitopes of foot-and-mouth disease virus. Agterberg, Marja; Adriaanse, Henriette; Lankhof, Hanneke; Meloen, Rob; Tommassen, Jan (Inst. Mol. Biol. Med. Biotechnol., Univ. Utrecht, Utrecht, 3584-CH,

Neth.). Vaccine, 8(1), 85-91 (English) **1990**. CODEN: VACCDE.
ISSN: 0264-410X.
AB Outer membrane protein PhoE of **E. coli** was used for the expression of antigenic determinants of foot-and-mouth disease virus. Five hybrid PhoE proteins were constructed containing different combinations of 2 antigenic determinants of VP1 protein of the virus. The hybrid proteins were expressed in 2 **E. coli** strains and the proteins were correctly assembled into the outer membrane. The inserted epitopes were exposed at the surface of the cell and were antigenic in this PhoE-associated conformation. Immunization expts., performed with partially purified protein, resulted in all cases in a significant anti-peptide antibody titer. In one case in which the hybrid protein with the largest insert was used, a neutralizing antibody response was detected.

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L7 16 L1 AND ALLERGEN

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L8 ANSWER 1 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
2007:576104 Document No.: PREV200700573458. Novel recombinant interleukin-13 peptide-based vaccine reduces airway allergic inflammatory responses in mice. Ma, Yanbing; HayGlass, Kent T.; Becker, Allan B.; Fan, Yijun; Yang, Xi; Basu, Sujata; Srinivasan, Ganesh; Estelle, F.; Simons, R.; Halayko, Andrew J.; Peng, Zhikang [Reprint Author]. Univ Manitoba, Dept Pediat and Child Hlth, 715 McDermot Ave, Winnipeg, MB R3E 3P4, Canada. zpeng@ms.umanitoba.ca. American Journal of Respiratory and Critical Care Medicine, (SEP 1 2007) Vol. 176, No. 5, pp. 439-445.
ISSN: 1073-449X. Language: English.
AB Rationale: Interleukin (IL)-13 plays a pivotal role in the pathogenesis of allergic asthma. Passive administration of its monoclonal antibody or soluble receptor to block overproduced IL-13 has been proven to be effective in controlling airway allergic responses in animal models, but these approaches have disadvantages of short half-lives, high costs, and possible adverse effects.Objectives: We sought to develop a novel therapeutic strategy through constructing an IL-13 peptide-based vaccine for blocking IL-13 on a persistent effect basis and to evaluate its in vivo effects using a murine model.Methods: To break self-tolerance, truncated hepatitis B core antigen was used as a carrier. Vaccine was prepared by inserting a peptide derived from the receptor binding site of mouse IL-13 into the immunodominant epitope region of the carrier using gene recombination methods. Mice received vaccine subcutaneously three times, and then subjected to intraperitoneal sensitization and intranasal challenge with ovalbumin. Control animals received carrier or saline in place of vaccine.Measurements and Main Results: The vaccine presented as virus-like particles and induced sustained and high titered IL-13-specific IgG without the use of conventional adjuvant. Vaccination significantly suppressed ovalbumin-incluced inflammatory cell number, and IL-13 and IL-5 levels in bronchoalveolar lavage fluids. Serum total and ovalbumin-specific IgE were also significantly inhibited. Moreover, **allergen**-induced goblet cell hyperplasia, lung tissue inflammatory cell infiltration, and pulmonary hyperresponsiveness to inhaled methacholine were significantly suppressed in vaccinated mice.Conclusions: Our data indicate that IL-13 peptide-based vaccines could be an effective therapeutic approach in the treatment of asthma.

L8 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
2006:169866 Document No. 144:187529 Carrier for carrying out functional tests on biological cells and method for coating said carrier. Steuer, Heiko; Templin, Markus; Kanzok, Britta; Kuschel, Cornelia; Angres, Brigitte (NMI Naturwissenschaftliches und Medizinisches Institut An der Universitaet Tuebingen, Germany). PCT Int. Appl. WO 2006018072 A1 20060223, 42 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (German). CODEN: PIXXD2. APPLICATION: WO 2005-EP7334 20050707. PRIORITY: DE 2004-102004039628 20040810.

AB The invention relates to a method for coating a carrier for carrying out functional tests on biol. cells, to a carrier for carrying out functional tests on biol. cells and to the use of corresponding carriers for carrying out said tests. The process involves (a) the coating of a carrier plate with a hydrogen-bridge donor and/or at least a polycationic substance; (b) applying onto the first layer a hydrogen-bridge acceptor and/or at least a polyanion; (c) immobilizing biomols. and test substances onto the coated layer; (d) incubation with a protein solution; (e) immobilization of cells onto the pretreated carrier. The carrier is glass, plastics, especially polystyrene and/or silicone. Layer (a) is selected from the group of poly-L-lysine, poly-D-lysine, polyamide, aminosilane or their derivs. Layer (b) is nitrocellulose that is applied from a methanolic solution by dipping or spraying. Proteins, especially extracellular matrix proteins, carbohydrates, glycoseaminoglycans, proteoglycans, and lipids are the immobilized biomols. Test substances are antibodies, drugs, messenger mols., growth factors, antigens, and **allergens**. Test substances are applied by impact or nonimpact printing using a printing buffer with trehalose.

L8 ANSWER 3 OF 8 MEDLINE on STN DUPLICATE 1
2005156208. PubMed ID: 15787872. Why Chlamydia pneumoniae is associated with asthma and other chronic conditions? Suggestions from a survey in unselected 9 yr old schoolchildren. Ronchetti Roberto; Biscione Gian Luca; Ronchetti Francesco; Ronchetti Maria Paola; Martella Susy; Falasca Carlo; Casini Carolina; Barreto Mario; Villa Maria Pia. (Pediatric Clinic, Second School of Medicine, Sant'Andrea Hospital, University La Sapienza, Rome, Italy.. ronchetti@uniroma1.it) . Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology, (2005 Mar) Vol. 16, No. 2, pp. 145-50. Journal code: 9106718. ISSN: 0905-6157. Pub. country: England: United Kingdom. Language: English.

AB Despite numerous studies demonstrating an association between asthma and many other chronic conditions and signs of Chlamydia pneumoniae (Cp) infection, the role of Cp in the pathogenesis of these illness remain still unclear. We investigated the prevalence of Cp antigen in the upper airways and the prevalence of detectable Cp serum antibodies in an unselected population of 207 9-yr-old schoolchildren. We also sought the presence of asthma, chronic or recurrent respiratory symptoms by means of questionnaire completed by the parents. Nasal aspirate, blood sampling and **allergen** skin prick tests were also performed. None of the children had obvious signs of acute infection at physical examination. Cp DNA was detected in nasal aspirates from 20 of the 207 children tested and serum IgG antibodies for Cp in 68 children. No association was found between atopy or history of atopic illness and the presence of Cp DNA or antibody production. This finding is explained by the fact that our study was conducted in an unselected childhood population, inherently including few children with asthma. A strong association between the status of

antigen carrier and the presence of detectable Cp serum immunoglobulin (Ig)G or IgM suggests that subjects with detectable Cp antibodies have an impaired ability to eliminate this pathogen when infected. Because Cp eradication requires a strong Th1 lymphocyte response, the previously proven association between Cp and asthma, might reflect the known association of asthma with Th2-oriented lymphocytic activity.

L8 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
1992:632030 Document No. 117:232030 Suppression of immune responses with oligomeric forms of antigen of controlled chemistry. Dintzis, Howard M.; Dintzis, Renee Z.; Blodgett, James K.; Cheronis, John C.; Kirschenheuter, Gary (Johns Hopkins University, USA). PCT Int. Appl. WO 9211029 A1 19920709, 230 pp. DESIGNATED STATES: W: AU, CA, JP, KR; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US9176 19911217. PRIORITY: US 1990-628858 19901217.
AB A method is provided of specifically suppressing an undesired immune response in a mammal suffering from such a response. The method comprises (1) preparing a construct comprising ≥1 discrete antigenically recognizable moiety (corresponding to a determinant of an antigen causing the undesired immune response) bound to a pharmacol. acceptable carrier, wherein the number of moieties bound to the carrier and the spacing of the moieties on the carrier are such that the construct does not elicit an immune response to the moieties but does directly compete with the antigen for receptors on an immunocompetent cell that recognizes the determinant, the construct thereby specifically suppressing the undesired immune response; and (2) administration of the construct to the mammal in an effective amount Also disclosed are methods for preparing the constructs (scaffold synthesis, conjugate preparation, etc.). A conjugate of dextran with a peptide derivative of a histone H2B amino-terminal fragment was prepared Anti-histone antibody titers in mice that received the suppressive conjugate were suppressed to background levels, while animals receiving control conjugates showed no significant changes (or, in many cases, actual increases) in their anti-histone antibody levels. Animals treated with immunosuppressive conjugate had no detectable cells actively secreting anti-histone antibodies, while control animals had a population of anti-histone antibody-secreting cells too numerous to quantitate. Immunogenicity of a variety of other constructs (e.g. fluoresceinated polymers, benzoylpenicillin conjugate with albumin or with ovalbumin) was examined

L8 ANSWER 5 OF 8 MEDLINE on STN DUPLICATE 2
90187904. PubMed ID: 2138201. A method to generate antigen-specific suppressor T cells in vitro from peripheral blood T cells of honey bee venom-sensitive, allergic patients. Carini C; Iwata M; Warner J; Ishizaka K. (Department of Medicine, Johns Hopkins University School of Medicine, Good Samaritan Hospital, Baltimore, MD 21239.) Journal of immunological methods, (1990 Mar 9) Vol. 127, No. 2, pp. 221-33. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB Peripheral blood mononuclear cells of patients allergic to honey bee venom were stimulated with denatured bee venom phospholipase A2, and the antigen-activated T cells were propagated for 4 days by human IL-2 in the presence or absence of recombinant human lipocortin I. Upon antigenic stimulation with the denatured phospholipase A2 and autologous monocytes or by cross-linking of CD3 by anti-CD3 antibody, the activated T cells, which had propagated by IL-2 alone, formed N-glycosylated IgE-binding factors and glycosylation enhancing factor (GEF), while those propagated in the presence of lipocortin formed unglycosylated IgE-binding factors and glycosylation inhibiting factor (GIF). The GEF and GIF formed by the antigen- or anti-CD3-stimulated T cells had affinity for bee venom phospholipase A2 and could be purified by using anti-lipomodulin

Sepharose. In the mouse lymphocyte system, the major cell source of GIF is antigen-specific suppressor T cells, and the antigen-binding GIF from the cells suppressed the in vivo antibody response in an **antigen** (**carrier**)-specific manner. In view of the findings in the mouse system, the present results may provide an immunological maneuver to generate **allergen**-specific suppressor T cells, and to obtain **allergen**-specific suppressor factor from T cell populations in the peripheral blood of allergic patients.

L8 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
1987:38519 Document No. 106:38519 Original Reference No. 106:6356h,6357a Preparation for the specific modification of the humoral or cellular immune reaction. Theurer, Karl (Fed. Rep. Ger.). Ger. Offen. DE 3513572 A1 19861016, 17 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1985-3513572 19850416.
AB Title prepns. used as carriers to the immune cells, natural soluble antigens, haptenes and **allergens**, or biomimetically-active antiidiotypic antibodies (European Patent 85102586.6) or sep. or conjugate antideterminant fragments thereof. The carriers can be used for immunosuppression or generation of immuno-tolerance by selective binding of cytotoxic, antimetabolic or alkylating agents, folic acid antagonists, dimeric alkaloids, radionuclides, antihistaminics, etc. The carriers can also be used for specific stimulation of the immune system by selective binding of juvenile lymph node, thymus, bone marrow or spleen exts., informative RNAs of DNAs for antibody formation, etc. Prepns. obtained with this carrier can be used for treatment of multiple sclerosis, myasthenia gravis, post-transplant tissue rejection, etc. Thus, a preparation for the specific immunosuppression of humoral or cellular autosensitization in multiple sclerosis uses as a carrier encephalogenic protein (Kibler, R. F. and Shapira, R., 1968) and myelin from the peripheral nervous system. Protein synthesis-inhibiting agents (erythromycin, chloramphenicol) are adsorbed or bound to the above carrier.

L8 ANSWER 7 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1985:339407 Document No.: PREV198580009399; BA80:9399. ENZYME IMMUNOASSAY USING POLYSTYRENE BEADS IN THE DIAGNOSIS OF JAPANESE CEDAR POLLINOSIS. MATSUI S [Reprint author]; NAKAZAWA T; INAZAWA M; UMEGAE Y; KOBAYASHI S; KOCHIBE N; SATO K. FIRST DEP OF INTERNAL MEDICINE, SCHOOL OF MEDICINE, GUNMA UNIVERSITY. Japanese Journal of Allergology, (1985) Vol. 34, No. 1, pp. 6-14.
CODEN: ARERAM. ISSN: 0021-4884. Language: JAPANESE.
AB An enzyme immunoassay (EIA) using polystyrene beads as an **antigen carrier** and β-D-galactosidase as the enzyme marker was developed for measuring specific IgE antibodies semiquantitatively in Japanese cedar pollinosis. Polystyrene beads can be well coated with the **allergen** extract from Japanese cedar pollen; ≥ 50 μg protein/ml of **allergen** is needed for the coating. Reproducibility of this method is acceptable because there was little difference in the results from each bead. Specific IgE antibody levels measured by this method correlated well with those by the radioallergosorbent test (RAST) using paper discs. Inhibition tests using antigen-coated beads and absorbed sera with various kinds of antigens showed that this method is also useful in the detection of activities of fractionated antigens. These results suggest that EIA using polystyrene beads is of value, in place of RAST, in isolation studies of **allergen** as well as in the diagnosis of Japanese cedar pollinosis.

L8 ANSWER 8 OF 8 MEDLINE on STN DUPLICATE 3
83292513. PubMed ID: 6604105. A nylon ball solid-phase radioimmunoassay for specific antibodies in human sera. Application to measurement of IgG antibodies to pollen **allergens**. Djurup R; Sondergaard I; Minuva

U; Weeke B. Journal of immunological methods, (1983 Sep 16) Vol. 62, No. 3, pp. 283-96. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The principle of the radioallergosorbent test (RAST) has been used to measure IgG antibodies to timothy grass pollen **allergens** in sera from desensitized allergic subjects. 125I-labeled goat anti-human IgG was used as detector protein. Non-specific binding was eliminated by use of a non-porous nylon ball an **antigen carrier** and by use of a special buffer with high ionic strength and pH, containing 1% bovine gamma globulin and 5% normal rabbit serum as 'balance proteins'. At dilution 1:80 non-specific binding was only 0.28% and the binding ratio for a high-liter serum was about 10. By inhibition experiments the assay was demonstrated to be specific for IgG antibodies to timothy grass pollen. The results obtained with this assay correlated statistically significantly with those found th a double -antibody method (rs equal 0.68, n equal 20, t equal 3.93, P less than 0.001). Serum dilution curves were parallel, indicating that the assay is in **allergen** excess. The within-assay coefficient of variation ranged from 3.9 to 7.6%; the between-assay coefficient of variation from 8.4 to 19.5%. The assay is very simple to perform, requiring no centrifugation. The **allergen** -coated balls are stable for at least 3 months. The assay should be applicable to measurement of IgG antibodies and IgG subclass antibodies to any protein antigen of interest.

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=> s antigen delivery
L9        3031 ANTIGEN DELIVERY

=> s l9 and "E Coli"
L10         89 L9 AND "E COLI"

=> s l10 and killed
L11          5 L10 AND KILLED

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12           3 DUP REMOVE L11 (2 DUPLICATES REMOVED)

=> d l12 1-3 cbib abs

L12  ANSWER 1 OF 3  BIOSIS  COPYRIGHT (c) 2008 The Thomson Corporation  on STN
2003:530572  Document No.: PREV200300533511. Delivery of antigens to the
     cytosol of nonprofessional phagocytic cells using invasive Escherichia
     coli K-12 expressing listeriolysin O. Pratt, J. T. [Reprint Author];
     Higgins, D. E. [Reprint Author]. Harvard Medical School, Boston, MA, USA.
     Abstracts of the General Meeting of the American Society for Microbiology,
     (2003) Vol. 103, pp. E-109. http://www.asmusa.org/mtgsrc/generalmeeting.ht
     m. cd-rom.
     Meeting Info.: 103rd American Society for Microbiology General Meeting.
     Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.
     ISSN: 1060-2011 (ISSN print). Language: English.
AB   Bacterial-based vectors can be used as vehicles for delivery of proteins
     to eukaryotic host cells.  The use of these vectors provides a distinct
     advantage because any protein that can be expressed by the bacteria can
     potentially be delivered without purification.  It has previously been
     shown that Escherichia coli expressing cytoplasmic recombinant
     listeriolysin O (LLO) can deliver antigenic protein to the cytosol of
     macrophages for processing and presentation through the MHC class I
     pathway.  LLO is a pore-forming cytolysin and an essential pathogenic
     determinant of Listeria monocytogenes.  However, the E.
     coli/LLO delivery system has been limited to use in professional
     phagocytic cells (macrophages and dendritic cells).  Therefore, we have
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modified the **E. coli**/LLO system to expand the delivery targets to nonprofessional phagocytic cells using an invasive strain of **E. coli**. We co-expressed LLO and invasin, an outer membrane protein from Yersinia pseudotuberculosis that mediates bacterial invasion through binding of host cell beta1 integrins, in an **E. coli** strain auxotrophic for peptidoglycan synthesis. Consequently, the **E. coli** induce phagocytosis through invasin-beta1 integrin interactions. Once inside the phagosome, bacteria undergo spontaneous lysis, releasing LLO and target proteins into the phagosome. Subsequent perforation of the phagosome by LLO allows the release of target proteins into the cytosol of host cells. Using T-cell activation assays, we have shown that both live and formalin-**killed E. coli** can efficiently deliver cytotoxic T-lymphocyte (CTL) antigens to the MHC class I presentation pathway of nonprofessional phagocytic cells. Furthermore, we have found that use of a peptidoglycan auxotroph is not required for efficient **antigen delivery**. In additional T-cell activation assays, **E. coli** without defects in peptidoglycan synthesis delivered antigen with approximately the same efficiency This system may facilitate the delivery of macromolecules to a wide variety of host cells and has the potential to be used as a delivery vector in a number of in vivo applications, including CTL stimulating vaccines and gene therapy.

L12 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2008 ACS on STN
1999:243466 Document No. 131:43311 Delivery of protein to the cytosol of macrophages using Escherichia coli K-12. Higgins, Darren E.; Shastri, Nilabh; Portnoy, Daniel A. (Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720, USA). Molecular Microbiology, 31(6), 1631-1641 (English) 1999. CODEN: MOMIEE. ISSN: 0950-382X. Publisher: Blackwell Science Ltd..
AB Listeriolysin O (LLO) is an essential determinant of pathogenicity whose natural biol. role is to mediate lysis of Listeria monocytogenes containing phagosomes. In this study, we report that Escherichia coli expressing cytoplasmic recombinant LLO can efficiently deliver co-expressed proteins to the cytosol of macrophages. We propose a model in which subsequent or concomitant to phagocytosis the **E. coli** are **killed** and degraded within phagosomes causing the release of LLO and target proteins from the bacteria. LLO acts by forming large pores in the phagosomal membrane, thus releasing the target protein into the cytosol. Delivery was shown to be rapid, within minutes after phagocytosis. Using this method, a large enzymically active protein was delivered to the cytosol. Furthermore, we demonstrated that the **E. coli**/LLO system is very efficient for delivery of ovalbumin (OVA) to the major histocompatibility (MHC) class I pathway for antigen processing and presentation, greater than 4 logs compared with **E. coli** expressing OVA alone. Moreover, the time required for processing and presentation of an OVA-derived peptide was similar to that previously reported when purified OVA was introduced directly into the cytosol by other methods. Using this system, potentially large amts. of any protein that can be expressed in **E. coli** can be delivered to the cytosol without protein purification The potential use of this system for the delivery of antigenic protein in vivo and the delivery of DNA are discussed.

L12 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
1998020885. PubMed ID: 9382741. Bacterial **antigen delivery** systems: phagocytic processing of bacterial antigens for MHC-I and MHC-II presentation to T cells. Svensson M; Pfeifer J; Stockinger B; Wick M J. (Dept. of Cell and Molecular Biology, Lund University, Sweden.) Behring Institute Mitteilungen, (1997 Feb) No. 98, pp. 197-211. Ref: 49. Journal code: 0367532. ISSN: 0301-0457. Pub.

country: GERMANY: Germany, Federal Republic of. Language: English.

AB Using an in vitro model system we have studied parameters of both bacteria and antigen presenting cells that influence peptide presentation by murine major histocompatibility complex class II (MHC-II) and class I (MHC-I) molecules. To study MHC-II presentation, the HEL (52-61) epitope, which binds the murine MHC-II molecule I-Ak, was expressed as the cytoplasmic Crl-HEL fusion protein in S. typhimurium. When murine peritoneal macrophages mediated phagocytic processing of S. typhimurium expressing Crl-HEL, HEL (52-61) was processed and presented on I-Ak more efficiently from heat-**killed** S. typhimurium than from viable bacteria, and from a rough LPS strain compared to its isogenic smooth LPS counterpart, most likely due to enhanced phagocytosis of the rough LPS strain. Macrophages also processed phoP S. typhimurium strains with greater efficiency for peptide presentation by I-Ak than wild type bacteria while Salmonella constitutively expressing phoP were processed for peptide presentation by I-Ak less efficiently than wild type Salmonella. We have also shown that macrophage phagocytosis of **E. coli** or S. typhimurium results in presentation of bacterial antigens by MHC-I molecules. To investigate the role of post-Golgi MHC-I molecules in this presentation pathway, peritoneal macrophages from TAP1-/- mice, which are deficient in presenting endogenous antigens on MHC-I and lack significant surface MHC-I expression, were co-incubated with bacteria containing the 257-264 epitope from ovalbumin [OVA(257-264)], which binds the murine class I molecule Kb. Peritoneal macrophages from TAP1-/-/ mice could process bacteria expressing the OVA epitope for recognition by epitope-specific T hybridoma cells. This processing and presentation was reduced in efficiency between three to 100 fold compared to C57BL/6 macrophages, depending on the protein harbouring the OVA (257-264) epitope (Crl-OVA or native OVA). This suggests that the protein context of the OVA (257-264) epitope influences the extent of TAP-independent processing for MHC-I presentation. In addition, we show that murine bone marrow-derived dendritic cells can phagocytose and process viable gram negative bacteria for peptide presentation on MHC-I and MHC-II; inhibition studies showed that acidic compartments in dendritic cells are required for this presentation. These results suggest that dendritic cells may be potential antigen presenting cells used in eliciting specific immune responses against bacteria.

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=> s l10 and allergen
L13          1 L10 AND ALLERGEN

=> d l13 cbib abs
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L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN

2003:610197 Document No. 139:148468 Methods and composition for delivering nucleic acids and/or proteins to the respiratory system. Chen, Wei; Fu, Xiaoli; Nouraini, Sherry; Zhang, Zhiqing (Symbigene, Inc., USA). PCT Int. Appl. WO 2003063786 A2 20030807, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US2469 20030127. PRIORITY: US 2002-353885P 20020131; US 2002-353923P 20020131; US 2002-401465P 20020805; US 2002-280769 20021025.

AB Methods and compostions related to the fields of bacteriol., immunol. and gene therapy are provided. In general modified microflora for the delivery of vaccines, **allergens** and therapeutics to the mucosal

surfaces of the respiratory tract are provided. In particular, the compns. and methods are directed at inducing an M-cell mediated immune response to pathogenic diseases. Specifically, methods of vaccine preparation, delivery and mucosal immunization using a Lactic Acid Bacteria (LAB), yeast and LAB that have been modified through fusion with **E. coli** to either present on its cell surface, or secrete, antigenic epitopes derived from pathogenic microorganisms and/or to secrete a therapeutic protein sequence are disclosed.

=> s l10 and modified alleren
L14 0 L10 AND MODIFIED ALLEREN

=> s l10 and allergen
L15 1 L10 AND ALLERGEN

=> d l15 cbib abs

L15 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
2003:610197 Document No. 139:148468 Methods and composition for delivering nucleic acids and/or proteins to the respiratory system. Chen, Wei; Fu, Xiaoli; Nouraini, Sherry; Zhang, Zhiqing (Symbigene, Inc., USA). PCT Int. Appl. WO 2003063786 A2 20030807, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US2469 20030127. PRIORITY: US 2002-353885P 20020131; US 2002-353923P 20020131; US 2002-401465P 20020805; US 2002-280769 20021025.
AB Methods and compostions related to the fields of bacteriol., immunol. and gene therapy are provided. In general modified microflora for the delivery of vaccines, **allergens** and therapeutics to the mucosal surfaces of the respiratory tract are provided. In particular, the compns. and methods are directed at inducing an M-cell mediated immune response to pathogenic diseases. Specifically, methods of vaccine preparation, delivery and mucosal immunization using a Lactic Acid Bacteria (LAB), yeast and LAB that have been modified through fusion with **E. coli** to either present on its cell surface, or secrete, antigenic epitopes derived from pathogenic microorganisms and/or to secrete a therapeutic protein sequence are disclosed.

=> s l9 and modified allergen
L16 0 L9 AND MODIFIED ALLERGEN

=> s allergen delivery
L17 60 ALLERGEN DELIVERY

=> s l17 and "E Coli"
L18 0 L17 AND "E COLI"

=> s l17 and bacteria
L19 1 L17 AND BACTERIA

=> d l19 cbib abs

L19 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
2001:676622 Document No. 135:225857 Microbial delivery system. Caplan,

Michael (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.

AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to allergens or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce allergens and protect the subjects from exposure to the allergens until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. **bacteria**, gram-pos. **bacteria**, and yeast. Particularly preferred allergens are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

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=> s allergen
L20     155308 ALLERGEN

=> s l20 and reduced IgE binding
L21        255 L20 AND REDUCED IGE BINDING

=> s l21 and bacteria
L22          6 L21 AND BACTERIA

=> dup remove l22
PROCESSING COMPLETED FOR L22
L23          6 DUP REMOVE L22 (0 DUPLICATES REMOVED)

=> d l23 1-6 cbib abs
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L23 ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

2008:70107 Document No.: PREV200800062051. A hypoallergenic vaccine obtained by tail-to-head restructuring of timothy grass pollen profilin, Phl p 12, for the treatment of cross-sensitization to profilin. Westritschnig, Kerstin; Linhart, Birgit; Focke-Tejkl, Margarete; Pavkov, Tea; Keller, Walter; Ball, Tanja; Mari, Adriano; Hartl, Arnulf; Stoecklinger, Angelika; Scheiblhofer, Sandra; Thalhamer, Josef; Ferreira, Fatima; Vieths, Stefan; Vogel, Lothar; Boehm, Alexandra; Valent, Peter; Valenta, Rudolf [Reprint Author]. Med Univ Vienna, Vienna Gen Hosp, Ctr Physiol and Pathophysiol, Dept Pathol,Christian Doppler Lab Allergy Res, Wahringer Gurtel 18-20, A-1090 Vienna, Austria. Rudolf.valenta@meduniwien.ac.at. Journal of Immunology, (DEC 1 2007) Vol. 179, No. 11, pp. 7624-7634. CODEN: JOIMA3. ISSN: 0022-1767. Language: English.

AB Profilins are highly cross-reactive **allergens** in pollens and plant food. In a paradigmatic approach, the cDNA coding for timothy grass pollen profilin, Phl p 12, was used as a template to develop a new strategy for engineering an allergy vaccine with low IgE reactivity. Non-IgE-reactive fragments of Phl p 12 were identified by synthetic peptide chemistry and restructured (rs) as a new molecule, Phl p 12-rs.

It comprised the C terminus of Phl p 12 at its N terminus and the Phl p 12 N terminus at its C terminus. Phl p 12-rs was expressed in Escherichia coli and purified to homogeneity. Determination of secondary structure by circular dichroism indicated that the restructuring process had reduced the IgE-reactive alpha-helical contents of the protein but retained its beta-sheet conformation. Phl p 12-rs exhibited **reduced IgE binding** capacity and allergenic activity but preserved T cell reactivity in allergic patients. IgG Abs induced by immunization of mice and rabbits with Phl p 12-rs cross-reacted with pollen and food-derived profilins. Recombinant Phl p 12-rs, rPhl p 12-rs, induced less reaginic IgE to the wild-type **allergen** than rPhl p 12. However, the rPhl p 12-rs-induced IgGs inhibited allergic patients' IgE Ab binding to profilins to a similar degree as those induced by immunization with the wild type. Phl p 12-rs specific IgG inhibited profilin-induced basophil degranulation. In conclusion, a restructured recombinant vaccine was developed for the treatment of profilin-allergic patients. The strategy of tail-to-head reassembly of hypoallergenic **allergen** fragments within one molecule represents a generally applicable strategy for the generation of allergy vaccines.

L23 ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

2007:243317 Document No.: PREV200700234468. Generation of a low Immunoglobulin E-binding mutant of the timothy grass pollen major **allergen** Phl p 5a. Wald, M. [Reprint Author]; Kahlert, H.; Weber, B.; Jankovic, M.; Keller, W.; Cromwell, O.; Nandy, A.; Fiebig, H.. Allergopharma J Ganzer KG, Res and Dev, Hermann Korner Str 52, D-21465 Reinbek, Germany. martin.wald@allergopharma.de. Clinical and Experimental Allergy, (MAR 2007) Vol. 37, No. 3, pp. 441-450.
ISSN: 0954-7894. Language: English.

AB Immunotherapy of grass pollen allergy is currently based on the administration of pollen extracts containing natural **allergens**. Specifically designed recombinant **allergens** with reduced IgE reactivity could be used in safer and more efficacious future therapy concepts.This study aimed to generate hypoallergenic variants of the timothy grass major **allergen** Phl p 5a as candidates for **allergen**-specific immunotherapy.Three deletion mutants were produced in Escherichia coli and subsequently purified. The overall IgE-binding capacity of the mutants was compared with the recombinant wild-type **allergen** by membrane blot and IgE-inhibition assays. The capacity for effector cell activation was determined in basophil activation assays. T cell proliferation assays with **allergen** -specific T cell lines were performed to confirm the retention of T cell reactivity. Structural properties were characterized by circular dichroism analysis and homogeneity by native isoelectric focusing. The deletion sites were mapped on homology models comprising the N- and C-terminal halves of Phl p 5a, respectively.The double-deletion mutant rPhl p 5a Delta(94-113, 175-198) showed strongly diminished IgE binding in membrane blot and IgE-inhibition assays. Both deletions affect predominantly alpha-helical regions located in the N- and C-terminal halves of Phl p 5a, respectively. Whereas deletion of Delta 175-198 alone was sufficient to cause a large reduction of the IgE reactivity in a subgroup of allergic sera, only the combination of both deletions was highly effective for all the sera tested. rPhl p 5a Delta(94-113, 175-198) consistently showed at least an 11.5-fold reduced capacity to activate basophils compared with the recombinant wild-type molecule, and the T cell proliferation assays demonstrated retention of T cell reactivity.The mutant rPhl p 5a Delta(94-113, 175-198) fulfils the basic requirements for a hypoallergenic molecule suitable for a future immunotherapy of grass pollen allergy; it offers substantially **reduced IgE binding** and maintained T cell reactivity.

L23 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

2001:2594 Document No.: PREV200100002594. Effects of proline mutations in the major house dust mite **allergen** Der f 2 on IgE-binding and histamine-releasing activity. Takai, Toshiro [Reprint author]; Ichikawa, Saori; Hatanaka, Hideki; Inagaki, Fuyuhiko; Okumura, Yasushi. Bioscience Research and Development Laboratory, Asahi Breweries, Ltd, 1-21, Midori 1-chome, Moriya-machi, Kitasoma-gun, Ibaraki, 302-0106, Japan. toshiro.takai@asahibeer.co.jp. European Journal of Biochemistry, (November, 2000) Vol. 267, No. 22, pp. 6650-6656. print. CODEN: EJBCAI. ISSN: 0014-2956. Language: English.

AB Der f 2 is the major group 2 **allergen** from house dust mite Dermatophagoides farinae and is composed of 129 amino-acid residues. Wild-type and six proline mutants of Der f 2 (P26A, P34A, P66A, P79A, P95A, and P99A) expressed in Escherichia coli were refolded and purified. Formations of intramolecular disulfide bonds in the purified proteins were confirmed correct. The apparent molecular masses analyzed by gel-filtration were 14-15 kDa. The IgE-binding capacity in the sera of seven mite-allergic patients, inhibitory activity for IgE-binding to immobilized wild-type Der f 2, and activity to stimulate peripheral blood basophils to release histamine in two volunteers were analyzed. P95A and P99A, which slightly differed from the wild-type Der f 2 in their CD spectrum, showed **reduced IgE-binding**, reduced inhibitory activity, and less histamine-releasing activity than the wild-type. P34A also showed reduced allergenicity. Considering that Pro95, Pro99 and Pro34 are closely located in loops at one end of the tertiary structure of Der f 2, we concluded that these loop regions included an IgE-binding site common to all tested patients. P66A showed **reduced IgE-binding** in two sera out of seven. P26A and P79A showed no reduced allergenicity. However, in immunoblot analysis after SDS/PAGE under reduced conditions, P79A showed no or markedly **reduced IgE-binding** while the other mutants showed IgE-binding corresponding to that in the assay using correctly refolded proteins. This suggests that Pro79 is involved in refolding of Der f 2. The findings in this study are important for the understanding of the antigenic structure of mite group 2 **allergens** and for manipulation of the **allergens** for specific immunotherapy.

L23 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN

1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been determined that **allergens**, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compound that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not

significantly altering or decreasing IgG binding capacity. The examples use peanut **allergens** to demonstrate alteration of IgE binding sites. The critical amino acids within each of the IgE binding epitopes of the peanut protein that are important to Ig binding have been determined Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most critical to IgE binding.

L23 ANSWER 5 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1997:500865 Document No.: PREV199799800068. Molecular characterization, expression in Escherichia coli, and epitope analysis of a two EF-hand calcium-binding birch pollen **allergen**, Bet v 4. Twardosz, Anna; Hayek, Brigitte; Seiberler, Susanne; Vangelista, Luca; Elfman, Lena; Gronlund, Hans; Kraft, Dietrich; Valenta, Rudolf [Reprint author]. Inst. Gen. Exp. Pathol., AKH, Univ. Vienna, Vienna, Austria. Biochemical and Biophysical Research Communications, (1997) Vol. 239, No. 1, pp. 197-204.
CODEN: BBRCA9. ISSN: 0006-291X. Language: English.
AB Birch pollen belongs to the most potent elicitors of Type I allergic reactions in early spring. Using serum IgE from a birch pollen allergic patient, two cDNA clones (clone 6 and clone 13) were isolated from a birch pollen expression cDNA library constructed in phage lambda-gt11. Clone 6 encoded a 9.3 kD two EF-hand calcium-binding protein, designated Bet v 4, with significant end to end sequence homology to EF-hand calcium-binding **allergens** from weed and grass pollen. Recombinant Bet v 4, expressed as beta-galactosidase fusion protein, reacted with serum IgE from approximately 20% of pollen allergic individuals. Depletion of **allergen**-bound calcium by EGTA treatment lead to a substantial reduction of IgE-binding to Bet v 4, indicating that protein-bound calcium is necessary for the maintenance of IgE-epitopes. The greatly **reduced IgE-binding** capacity of clone 13, a Bet v 4 fragment that lacked the 16 N-terminal amino acids, indicated that the N-terminus contributes significantly to the proteins IgE-binding capacity. By IgE-inhibition experiments it was demonstrated that recombinant Bet v 4 shared IgE-epitopes with natural Bet v 4 and a homologous timothy grass pollen **allergen**. Recombinant Bet v 4 may therefore be considered as a relevant crossreactive plant **allergen**, which may be used for diagnosis and treatment of patients suffering from multivalent plant allergies.

L23 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1994:526700 Document No.: PREV199497539700. Complementary DNA cloning of the major **allergen** PhI p I from timothy grass (Phleum pratense); recombinant PhI p I inhibits IgE binding to group I **allergens** from eight different grass species. Laffer, Sylvia; Valenta, Rudolf; Vrtala, Susanne; Susani, Markus; Van Ree, Ronald; Kraft, Dietrich; Scheiner, Otto; Duchene, Michael [Reprint author]. Inst. General Experimental Pathology, AKH, Waehringergeutel 18-20, A-1090 Vienna, Austria. Journal of Allergy and Clinical Immunology, (1994) Vol. 94, No. 4, pp. 689-698.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.
AB Background: Grass pollens, such as pollen from timothy grass (Phleum pratense), represent a major cause of type I allergy. Objective: In this report we attempted to determine how cross-reactive allergenic components of grass pollens from different species can be represented by a minimum number of recombinant **allergens**. Methods: We isolated and sequenced a timothy grass pollen cDNA coding for the major **allergen** Phl p I. A recombinant Phl p I-beta-galactosidase fusion protein, which bound to IgE in 87% of patients with grass pollen allergy, was produced in Escherichia coli. Using recombinant Phl p V and Phl p I, we defined representative patients' sera that bound to group I but not to group V **allergens**, as well as sera with reactivity against group

I and group V **allergens**. IgE immunoblot inhibition studies were done with nitrocellulose-blotted pollen extracts from eight grass species with different geographic distribution. Results: Preadsorption of patients' sera with recombinant nonfusion Phl p I strongly **reduced IgE binding** to group I **allergens** from the eight grasses. showing extensive cross-reactivity between species. Conclusion: A single recombinant group I **allergen** contains many of the IgE epitopes of group I isoallergens from a number of different grass species.

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L26 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2008 ACS on STN
2007:1320549 Document No. 148:142264 A Hypoallergenic Vaccine Obtained by Tail-to-Head Restructuring of Timothy Grass Pollen Profilin, Phl p 12, for the Treatment of Cross-Sensitization to Profilin. Westritschnig, Kerstin; Linhart, Birgit; Focke-Tejkl, Margarete; Pavkov, Tea; Keller, Walter; Ball, Tanja; Mari, Adriano; Hartl, Arnulf; Stoecklinger, Angelika; Scheiblhofer, Sandra; Thalhamer, Josef; Ferreira, Fatima; Vieths, Stefan; Vogel, Lothar; Boehm, Alexandra; Valent, Peter; Valenta, Rudolf (Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria). Journal of Immunology, 179(11), 7624-7634 (English) 2007. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.
AB Profilins are highly cross-reactive **allergens** in pollens and plant food. In a paradigmatic approach, the cDNA coding for timothy grass pollen profilin, Phl p 12, was used as a template to develop a new strategy for engineering an allergy vaccine with low IgE reactivity. Non-IgE-reactive fragments of Phl p 12 were identified by synthetic peptide chemical and restructured (rs) as a new mol., Phl p 12-rs. It comprised the C terminus of Phl p 12 at its N terminus and the Phl p 12 N terminus at its C terminus. Phl p 12-rs was expressed in **E. coli** and purified to homogeneity. Determination of secondary structure by CD indicated that the restructuring process had reduced the IgE-reactive α-helical contents of the protein but retained its β-sheet conformation. Phl p 12-rs exhibited **reduced IgE binding** capacity and allergenic activity but preserved T cell reactivity in allergic patients. IgG Abs induced by immunization of mice and rabbits with Phl p 12-rs cross-reacted with pollen and food-derived profilins. Recombinant Phl p 12-rs, rPhl p 12-rs, induced less reaginic IgE to the wild-type **allergen** than rPhl p 12. However, the rPhl p 12-rs-induced IgGs inhibited allergic patients' IgE Ab binding to profilins to a similar degree as those induced by immunization with the

wild type. Phl p 12-rs specific IgG inhibited profilin-induced basophil degranulation. Thus, a restructured recombinant vaccine was developed for the treatment of profilin-allergic patients. The strategy of tail-to-head reassembly of hypoallergenic **allergen** fragments within one mol. represents a generally applicable strategy for the generation of allergy vaccines.

L26 ANSWER 2 OF 3 MEDLINE on STN

94366422. PubMed ID: 7521933. Potential therapeutic recombinant proteins comprised of peptides containing recombined T cell epitopes. Rogers B L; Bond J F; Craig S J; Nault A K; Segal D B; Morgenstern J P; Chen M S; Bizinkauskas C B; Counsell C M; Lussier A M; +. (ImmuLogic Pharmaceutical Corporation, Waltham, MA 02154.) Molecular immunology, (1994 Sep) Vol. 31, No. 13, pp. 955-66. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The complete primary structure of Fel d I2 has been determined and shown to be comprised of two separate polypeptide chains (designated chain 1 and 2). Overlapping peptides covering the entire sequence of both chains of Fel d I have been used to map the major areas of human T cell reactivity. The present study describes three non-contiguous T cell reactive regions of < 30 aa in length that were assembled in all six possible configurations using PCR and recombinant DNA methods. These six recombinant proteins comprised of defined non-contiguous T cell epitope regions artificially combined into single polypeptide chains have been expressed in **E. coli**, highly purified, and examined for their ability to bind to human cat-allergic IgE and for human T cell reactivity. Several of these recombined T cell epitope-containing polypeptides exhibit markedly **reduced IgE binding** as compared to the native Fel d I. Importantly, the human T cell reactivity to individual T cell epitope-containing regions is maintained even though each was placed in an unnatural position as compared to the native molecule. In addition, T cell responses to potential junctional epitopes were not detected. It was also demonstrated in mice that s.c. injection of T cell epitope-containing polypeptides inhibits the T cell response to the individual peptides upon subsequent challenge in vitro. Thus, these recombined T cell epitope-containing polypeptides, which harbor multiple T cell reactive regions but have significantly reduced reactivity with allergic human IgE, constitute a novel potential approach for desensitization to important **allergens**.

L26 ANSWER 3 OF 3 MEDLINE on STN

93375976. PubMed ID: 8366858. Purification and immunochemical characterization of recombinant and native ragweed **allergen** Amb a II. Kuo M C; Zhu X J; Koury R; Griffith I J; Klapper D G; Bond J F; Rogers B L. (ImmuLogic Pharmaceutical Corporation, Waltham, MA.) Molecular immunology, (1993 Aug) Vol. 30, No. 12, pp. 1077-87. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The complete sequence of a cDNA encoding Amb a II and its relationship to the Amb a I family of **allergens** has recently been described [Rogers et al. (1991) J. Immun. 147, 2547-2552; Griffith et al. (1991a), Int. Archs Allergy appl. Immun. 96, 296-304]. In this study, we present results generated with rabbit antipeptide antisera that recognize Amb a II or Amb a I, but not both. The specificity of two anti-Amb a II antipeptide sera, anti-RAE-50.K and anti-RAE-51.K, was verified on Western blots of recombinant Amb a II and Amb aI.1. These two sera, directed against separate regions of the Amb a II molecule, detected three individual 38-kDa Amb a II isoforms on 2D Western blots of aqueous ragweed pollen extract. These Amb a II isoforms have pI in the 5.5-5.85 range and can be easily distinguished from Amb a I isoforms with pI in the 4.5-5.2 range detected by an anti-Amb a I specific peptide antiserum. The Amb a

II isoforms have also been individually purified from pollen, positively identified as Amb a II by amino acid sequencing, and visualized as separate bands on IEF gels. An analysis of Amb a II cDNA sequences generated by PCR led to the prediction of three Amb a II isoforms with pI of 5.74, 5.86 and 5.97 that are very similar to the pI deduced from 2D Western blot analysis. Recombinant Amb aI.1 and Amb a II have been expressed in **E. coli**, purified in their denatured form, and examined by ELISA for their capacity to bind pooled allergic human IgE. Purified native Amb a and Amb a II from pollen were shown to have very similar IgE-binding properties. In contrast, Amb a II had a markedly **reduced IgE-binding** capacity as compared to Amb a I.1. These data suggest that recombinant Amb a I.1 and Amb a II, isolated in a denatured form, differ significantly in their IgE-binding properties whereas the native molecules isolated from pollen do not.

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=> s "allergen delivery"
L27         60 "ALLERGEN DELIVERY"

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L28          2 L27 AND MUCOSAL

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L29           1 DUP REMOVE L28 (1 DUPLICATE REMOVED)

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L29  ANSWER 1 OF 1     MEDLINE on STN                    DUPLICATE 1
2003483255.    PubMed ID: 14561171.   Respiratory tolerance in the protection
     against asthma. Macaubas Claudia; DeKruyff Rosemarie H; Umetsu Dale T.
     (Division of Immunology and Allergy, Department of Pediatrics, Stanford
     University, Stanford, CA 94305-5208, USA.. macaubas@stanford.edu) .
     Current drug targets. Inflammation and allergy, (2003 Jun) Vol. 2, No. 2,
     pp. 175-86.  Ref: 121. Journal code: 101160019. ISSN: 1568-010X. Pub.
     country: Netherlands. Language: English.
AB   Understanding the pathways involved in the induction and maintenance of
     respiratory tolerance to airborne allergens is important in designing new
     therapies for asthma and other allergic diseases that not only control
     disease symptoms, but also change or potentially cure the disease.
     Respiratory tolerance, and mucosal immunity are maintained by a
     complex system of defense mechanisms.  Most of the inhaled environmental
     load is eliminated by exclusion mechanisms, which include physical
     barriers, such as mucus, and cilia as well as a variety of mediators with
     anti-microbial and immunomodulatory properties.  Blanket immunosuppression
     is provided by alveolar macrophages, which inhibit antigen presentation
     and T cell responses, in addition to their role in pathogen elimination.
     Furthermore, there is antigen specific unresponsiveness or tolerance.
     This tolerance is mediated by lung dendritic cells producing IL-10, which
     induce the development of CD4+ T regulatory cells.  The development of
     respiratory tolerance also depends on co-stimulation (CD86, and the
     ICOS-ICOSL pathway).  Although exposure of the respiratory mucosa to some
     pathogenic agents (especially virus, and endotoxin) is associated with
     asthma exacerbations, microbial exposure may also promote mucosal
     tolerance and protection against the development of allergic diseases, but
     the mechanisms involved are not very well understood.  Mucosal
     -based immunotherapy has been already used as an alternative form of
     allergen delivery in immunotherapy, the only available
     treatment that is able to reverse established allergic disease.
     Strategies to further improve mucosal immunotherapy include the
     use of modified allergen derived peptides, and adjuvants like CpG motifs.
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=> s l27 and bacteria
L30          1 L27 AND BACTERIA

=> d l30 cbib abs

L30  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2008 ACS on STN
2001:676622   Document No. 135:225857  Microbial delivery system.  Caplan,
     Michael (Panacea Pharmaceuticals, LLC, USA).  PCT Int. Appl. WO 2001066136
     A2 20010913, 57 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ,
     BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES,
     FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ,
     LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL,
     PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
     YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF,
     CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML,
     MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).  CODEN: PIXXD2.
     APPLICATION: WO 2000-US33121 20001206.  PRIORITY: US 2000-PV195035
     20000306.
AB   The present invention provides methods and compns. for treating or
     preventing allergic responses, particularly anaphylactic allergic
     responses, in subjects who are allergic to allergens or susceptible to
     allergies.  Methods of the present invention utilize administration of
     microorganisms to subjects, where the microorganisms produce allergens and
     protect the subjects from exposure to the allergens until phagocytosed by
     antigen-presenting cells.  Particularly preferred microorganisms are
     gram-neg. bacteria, gram-pos. bacteria, and yeast.
     Particularly preferred allergens are proteins found in foods, venoms,
     drugs and latex that elicit allergic reactions and anaphylactic allergic
     reactions in individuals who are allergic to the proteins or are
     susceptible to allergies to the proteins.  The proteins may also be
     modified to reduce the ability of the proteins to bind and crosslink IgE
     antibodies and thereby reduce the risk of eliciting anaphylaxis without
     affecting T-cell mediated Th1-type immunity.


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L32          0 L27 AND HEAT KILLED

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L34          1 L33 AND MICROBIAL DELIVERY

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L34  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2008 ACS on STN
2001:676622   Document No. 135:225857  Microbial delivery
     system.  Caplan, Michael (Panacea Pharmaceuticals, LLC, USA).
     PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU,
     CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN,
     IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
     MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
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(English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.

AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to allergens or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce allergens and protect the subjects from exposure to the allergens until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. bacteria, gram-pos. bacteria, and yeast. Particularly preferred allergens are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

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L35 10 L33 AND E COLI

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L36 0 L35 AND MODIFIED

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L37 3 DUP REMOVE L35 (7 DUPLICATES REMOVED)

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L37 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2008 ACS on STN

2005:259357 Document No. 142:334946 Recombinant allergens with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex allergens. **Caplan, Michael J.**; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 20050063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.

AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein allergen. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein allergen. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as **E. coli** . Any allergen may be used in the inventive methods. Particularly preferred allergens are anaphylactic allergens including protein allergens found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L37 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1

1999394992. PubMed ID: 10464133. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. **Caplan M S**; Miller-Catchpole R; Kaup S; Russell T; Lickerman M; Amer M; Xiao Y; Thomson R Jr. (Department of Pediatrics, Northwestern University Medical School, Evanston Hospital, Evanston, Illinois, USA.) Gastroenterology, (1999 Sep) Vol. 117, No. 3, pp. 577-83. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: Neonatal necrotizing enterocolitis (NEC) is a

devastating gastrointestinal disease of premature infants partly caused by intestinal bacterial proliferation. Because bifidobacteria are thought to reduce the risk for intestinal disturbances associated with pathogenic bacterial colonization, we hypothesized that exogenous bifidobacterial supplementation to newborn rats would result in intestinal colonization and a reduction in the incidence of neonatal NEC. METHODS: Newborn rat pups were given Bifidobacterium infantis (10(9) organisms per animal daily), Escherichia coli, or saline control and exposed to the NEC protocol consisting of formula feeding (Esbilac; 200 cal. kg(-1). day(-1)) and asphyxia (100% N(2) for 50 seconds followed by cold exposure for 10 minutes). Outcome measures included stool and intestinal microbiological evaluation, gross and histological evidence of NEC, plasma endotoxin concentration, intestinal phospholipase A(2) expression, and estimation of intestinal mucosal permeability. RESULTS: Bifidobacterial supplementation resulted in intestinal colonization by 24 hours and appearance in stool samples by 48 hours. Bifidobacteria-supplemented animals had a significant reduction in the incidence of NEC compared with controls and **E. coli**-treated animals (NEC, 7/24 B. infantis vs. 19/27 control vs. 16/23 **E. coli**; P < 0.01). Plasma endotoxin and intestinal phospholipase A(2) expression were lower in bifidobacteria-treated pups than in controls, supporting the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC. CONCLUSIONS: Intestinal bifidobacterial colonization reduces the risk of NEC in newborn rats.

L37 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 2
95073279. PubMed ID: 7982271. Altered mitochondrial redox responses in gram negative septic shock in primates. Simonson S G; Welty-Wolf K; Huang Y T; Griebel J A; **Caplan M S**; Fracica P J; Piantadosi C A. (Department of Medicine, Duke University Medical Center, Durham, NC 27710.) Circulatory shock, (1994 May) Vol. 43, No. 1, pp. 34-43. Journal code: 0414112. ISSN: 0092-6213. Pub. country: United States. Language: English.
AB Gram negative sepsis causes changes in oxygen supply-demand relationships. We have used a primate model of hyperdynamic gram negative sepsis produced by intravenous infusion of Escherichia coli (**E. coli**) to evaluate sepsis-induced alterations in mitochondrial oxidation-reduction (redox) state in muscle in vivo. The redox state of cytochrome a,a3, the terminal member of the intramitochondrial respiratory chain, was assessed in the intact forearm by near-infrared (NIR) spectroscopy. The muscle NIR data were compared to routine measures of oxygen delivery (DO2) and oxygen consumption (VO2). After **E. coli** infusion and fluid resuscitation, DO2 and VO2 showed minimal changes through 24 hr of sepsis. In contrast, changes in cytochrome a,a3 redox state evaluated by NIR occurred within a few hours and were progressive. Mitochondrial functional responses were correlated with structural changes observed on serial muscle biopsies. Gross morphological changes in muscle mitochondria were present in some animals as early as 12 hr, and, in most animals, by 24 hr. The morphologic changes were consistent with decreases in oxidative capacity as suggested by NIR spectroscopy. The NIR data also suggest that two mechanisms are operating to explain abnormalities in oxygen metabolism and mitochondrial function in lethal sepsis. These mechanisms include an early defect in oxygen provision to mitochondria that is followed by a progressive loss in functional cytochrome a,a3 in the muscle.

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L38 14 L33 AND ALLERGEN

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L39 14 DUP REMOVE L38 (0 DUPLICATES REMOVED)

=> d l39 1-14 cbib abs

L39 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2005:259357 Document No. 142:334946 Recombinant **allergens** with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex **allergens**. **Caplan, Michael J.**; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 20050063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.

AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein **allergen**. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein **allergen**. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as E. coli . Any **allergen** may be used in the inventive methods. Particularly preferred **allergens** are anaphylactic **allergens** including protein **allergens** found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L39 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food **allergens** with reduced IgE-binding ability for decreasing clinical reaction to allergy. **Caplan, Michael J.**; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 20030202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified **allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural **allergen**. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen**. In certain embodiments, modified **allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural **allergen**. In certain embodiments, the methods of the present invention allow **allergens** to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The

immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

L39 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food **allergens** with reduced IgE-binding ability for decreasing clinical reaction to allergy. **Caplan, Michael**; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.
AB It has been determined that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified **allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural **allergen**. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen**. In certain embodiments, modified **allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural **allergen**. In certain embodiments, the methods of the present invention allow **allergens** to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

L39 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2002:123512 Document No. 136:182453 IgE-blocking agents for passive desensitization. **Caplan, Michael J.** (USA). U.S. Pat. Appl. Publ. US 20020018778 A1 20020214, 22 pp., Cont.-in-part of U.S. Ser. No. 455,294. (English). CODEN: USXXCO. APPLICATION: US 2000-731221 20001206. PRIORITY: US 1999-455294 19991206; US 2000-213765P 20000623; US 2000-235797P 20000927.
AB IgE-blocking agents and methods of their use have been developed for desensitizing an individual to an antigen. These IgE-blocking agents work by blocking the antigen-binding site of the IgE mols. and thereby preventing the antigen from binding. These agents typically have up to one IgE binding site present per mol. so as prevent any crosslinking of IgE which could lead to an allergic reaction. The IgE-blocking agents include **allergen** epitope, antibody, or Ig. fragment. Methods of using these novel IgE blocking agents include administering the agents to

alleviate or prevent allergic reactions as well as administering the agents to decrease the risk of allergic reactions during immunotherapy or "rush" immunotherapy. The IgE-blocking agents may be combined with immune adjuvant or cytokine for treatment. Compns. and kits comprising these IgE binding agents are also provided.

L39 ANSWER 5 OF 14 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
2001:559717 Document No.: PREV200100559717. Methods to block IGE binding to cell surface receptors of mast cells. **Caplan, Michael** [Inventor]; Sosin, Howard [Inventor, Reprint author]. Fairfield, CT, USA. ASSIGNEE: Panacea Pharmaceuticals, LLC. Patent Info.: US 6299875 20011009. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 9, 2001) Vol. 1251, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB Compositions are administered to block IgE binding to receptors and ultimately displace native IgE from mast cells and related cell types, to prevent the activation of these cells during an allergic response. The compositions consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcepsilonRI IgE binding sites, and more preferably, FcepsilonRI and FcepsilonRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to **allergens**. The compounds can consist of IgE molecules and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-crosslinkable IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

L39 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2001:676622 Document No. 135:225857 Microbial delivery system. **Caplan, Michael** (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.
AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to **allergens** or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce **allergens** and protect the subjects from exposure to the **allergens** until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. bacteria, gram-pos. bacteria, and yeast. Particularly preferred **allergens** are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

L39 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2001:416973 Document No. 135:45198 Prevention of an anaphylactic response to food **allergens**. Bannon, Gary A.; Burks, Wesley A.; **Caplan, Michael J.**; Sampson, Hugh; Sosin, Howard (Panacea Pharmaceuticals, LLC, USA; University of Arkansas; Mount Sinai School of Medicine,

University of New York). PCT Int. Appl. WO 2001040264 A2 20010607, 100 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33124 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.

AB The authors disclose methods for reducing allergic responses in individuals sensitive to one or more food antigens. In general, desensitization is achieved by administration of fragments of antigens characterized by a reduced ability to bind to their cognate IgE. In one example, mice were sensitized to peanut **allergens** by intragastric feeding. Administration of peptide fragments of Ara h 2, or an **allergen** mutein with altered IgE binding sites, abrogated an increase in IgE levels and anaphylactic sequelae.

L39 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN

2001:416792 Document No. 135:10056 Controlled delivery of antigens. **Caplan, Michael**; Burks, Wesley A., Jr.; Bannon, Gary A. (The Board of Trustees of the University of Arkansas, USA; Panacea Pharmaceuticals, LLC). PCT Int. Appl. WO 2001039800 A2 20010607, 34 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US42607 20001206. PRIORITY: US 1999-PV169330 19991206.

AB Formulations and methods are developed for delivering antigens to individuals in a manner that substantially reduces contact between the antigen and IgE receptors displayed on the surfaces of cells involved in mediating allergic responses, which target delivery of antigen to dendritic, phagocytic and antigen presenting cells (APCs), and which have improved pharmacokinetics. By reducing direct and indirect association of antigens with antigen-specific IgE antibodies, the risk of an allergic reaction, possibly anaphylactic shock, is reduced or eliminated. Particularly preferred antigens are those that may elicit anaphylaxis in individuals, including food antigens, insect venom and rubber-related antigens. In the preferred embodiments, the compns. include one or more antigens in a delivery material such as a polymer, in the form of particles or a gel, or lipid vesicles or liposomes, any of which can be stabilized or targeted to enhance delivery. Preferably, the antigen is surrounded by the encapsulation material. Alternatively or addnl., the antigen is displayed on the surface of the encapsulation material. One result of encapsulating antigen is the reduction in association with antigen-specific IgE antibodies. In some embodiments, antigens are stabilized or protected from degradation until the antigen can be recognized and endocytized by APCs which are involved in eliciting cellular and humoral immune responses. In a preferred embodiment, the formulation is designed to deliver antigens to individuals in a manner designed to promote a Th1-type mediated immune response and/or in a manner designed to suppress a Th2 response. In still another embodiment, the formulation effects preferential release of the antigen within APCs. For example, various synthetic, biodegradable polymeric microsphere formulations were prepared containing peanut **allergen**. Microspheres based on poly(lactide-co-glycolide) (75:25) containing an acid end group (0.1% loaded

with **allergen**) had the lowest amount (<20 ng) of peanut protein detected on the outside of the microsphere and the best range of peanut protein **allergens** contained within the microspheres (having mol. wts. ranging from 15 kDa to 70 kDa).

L39 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2001:416791 Document No. 135:32734 Passive desensitization. **Caplan, Michael** (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001039799 A2 20010607, 76 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33125 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.
AB IgE-blocking agents and methods of their use have been developed for desensitizing an individual to an antigen. These IgE-blocking agents work by blocking the antigen-binding site of the IgE mols. and thereby preventing the antigen from binding. These agents typically have up to one IgE binding site present per mol. so as to prevent any crosslinking of IgE which could lead to an allergic reaction. Methods of using these novel IgE blocking agents include administering the agents to alleviate or prevent allergic reactions as well as administering the agents to decrease the risk of allergic reactions during immunotherapy or "rush" immunotherapy. Compns. and kits comprising these IgE binding agents are also provided.

L39 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2000:741936 Document No. 133:308997 Methods for skewing the balance between Th1 and Th2 immune responses. Bottomly, H. Kim; **Caplan, Michael J.**; Sosin, Howard B. (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2000061157 A1 20001019, 76 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9270 20000407. PRIORITY: US 1999-290029 19990409.
AB The present invention provides compns. and methods for regulating immune system reactions by biasing T cell responses away from Th1 or Th2 responses in a pre-determined manner. Control is effected at the stage of antigen/APC encounter and/or at the stage of APC/T cell encounter. In preferred embodiments, a Th1 or Th2 response is inhibited through induction of the alternative response. The inventive methods and reagents are particularly useful for the management of autoimmune disorders, allergy, and asthma.

L39 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
2000:666624 Document No. 133:251267 Immunostimulatory nucleic acids and antigens. Sosin, Howard B.; **Caplan, Michael J.** (Panacea Pharmaceuticals, Llc, USA). PCT Int. Appl. WO 2000054803 A2 20000921, 103 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB,

GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US7213 20000316. PRIORITY: US 1999-PV124595 19990316; US 1999-PV125071 19990317.

AB The present invention provides methods and compns. for modulating an individual's immune response to antigens. It is an aspect of the present invention that allergic responses to antigens, which in some cases lead to asthma and even anaphylaxis, can be treated or prevented by administering compns. having immunostimulatory oligonucleotides having unmethylated CpG sequences. It is another aspect of the present invention that allergies to antigens, especially one that result in asthma and anaphylaxis, can be treated or prevented by administering compns. containing immunostimulatory oligonucleotides having unmethylated CpG dinucleotide sequences and further comprising antigen(s), fragments of the antigen, mixts. of fragments of the antigen, antigens modified to reduce Th2-type immune responses, and fragments of the antigen modified to reduce Th2-type immune responses. Cellular systems for studying immunostimulation by CpG containing nucleic acids include in vivo, in vitro or ex vivo systems.

L39 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN

2000:573822 Document No. 133:163051 Method for altering immune responses to polypeptides. **Caplan, Michael** (Panacea Pharmaceuticals, Llc, USA). PCT Int. Appl. WO 2000047610 A2 20000817, 49 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US3448 20000210. PRIORITY: US 1999-247406 19990210.

AB The author discloses methodol. for altering undesirable immune responses to polypeptides by their recombinant engineering. Such polypeptides are safer and can be more efficacious when introduced into a human, other mammal, or other animal. The disclosed method involves providing a collection of mutant polypeptides where the amino acid sequence of each mutant polypeptide differs in at least one position from a polypeptide of interest. Mutant polypeptides that exhibit less of the immune response than the polypeptide of interest, but still retain desired characteristic(s) are then identified. The collection of mutant polypeptides is provided by mutagenizing nucleic acid encoding a polypeptide and expressing the mutagenized nucleic acid.

L39 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN

2000:420987 Document No. 133:57594 Decreasing allergic reactions by inhibition of IgE binding. **Caplan, Michael**; Sosin, Howard (USA). PCT Int. Appl. WO 2000035484 A2 20000622, 21 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US30238 19991217. PRIORITY: US 1998-216117 19981218.

AB The authors disclose methodol. for preventing allergic response by the inhibition of IgE binding to its epitopes on cognate **allergens**. Mols. which bind to these epitopes can be identified and synthesized and then formulated to coat or blend with the allergenic components to prevent IgE binding. In one example, the inhibitory mols. are IgE fragments selected using phage display technol. In a second example, the masking reagents are CDR-derived peptides or peptidomimetics which bind to the relevant epitopes on the **allergens**.

L39 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
1999:783962 Document No. 132:22180 Compounds binding specifically to FcεRI IgE binding sites for pan-specific anti-allergy therapy. **Caplan, Michael**; Sosin, Howard (USA). PCT Int. Appl. WO 9962550 A1 19991209, 28 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US12526 19990604. PRIORITY: US 1998-90375 19980604.

AB Compns. are administered to block IgE binding to receptors and ultimately displace native IgE from mast cells and related cell types, to prevent the activation of these cells during an allergic response. The compns. consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcεRI IgE binding sites, and more preferably, FcεRI and FcεRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to **allergens**. The compds. can consist of IgE mols. and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-cross-linkable IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

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NEWS 9	APR 07	MEDLINE Coverage Is Extended Back to 1947
NEWS 10	JUN 16	WPI First View (File WPIFV) will no longer be available after July 30, 2010
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NEWS 18	JUL 19	Enhancement of citation information in INPADOC databases provides new, more efficient competitor analyses
NEWS 19	JUL 26	CAS coverage of global patent authorities has expanded to 61 with the addition of Costa Rica

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FULL ESTIMATED COST                                   0.22       0.22

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=> s allergy vaccine
L1          686 ALLERGY VACCINE

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L2            5 L1 AND E COLI

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L3            5 DUP REMOVE L2 (0 DUPLICATES REMOVED)

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L3   ANSWER 1 OF 5  SCISEARCH  COPYRIGHT (c) 2010 The Thomson Corporation  on
     STN
2010:810053   The Genuine Article (R) Number: 621QI. Recombinant Fusion
     Proteins Assembling Der p 1 and Der p 2 Allergens from Dermatophagoides
     pteronyssinus.
     Moingeon, Philippe (Reprint). Stallergenes SA, Res & Dev, 6 Rue Alexis
     Tocqueville, FR-92183 Antony, France (Reprint). E-mail:
     pmoingeon@stallergenes.fr. Bussieres, Laetitia; Bordas-Le Floch,
     Veronique; Bulder, Ingrid; Chabre, Henri; Nony, Emmanuel; Lautrette,
     Aurelie; Berrouet, Christelle; Nguefeu, Yvette; Horiot, Stephane;
     Baron-Bodo, Veronique; Van Overtvelt, Laurence; De Conti, Anne Marie;
     Lemoine, Pierrick; Batard, Thierry; Moingeon, Philippe (Reprint).
     Stallergenes, Res & Dev, Antony, France. E-mail:
     pmoingeon@stallergenes.fr. Schlegel, Anne; Maguet, Nicolas; Mouz, Nicolas.
     PX Therapeut, Grenoble, France.
     INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (2010) Vol. 153, No. 2,
     pp. 141-151. ISSN: 1018-2438. Publisher: KARGER, ALLSCHWILERSTRASSE 10,
     CH-4009 BASEL, SWITZERLAND. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        Background: Fusion proteins assembling multiple allergens can be
     engineered by recombinant DNA technologies in order to produce tools for
     diagnostic and immunotherapeutic purposes.  Herein, we developed and
     characterized chimeras assembling Der p 1 and Der p 2 allergens as
     potential candidate vaccines against house dust mite allergy.  Methods:
     Fusion proteins encompassing Der p 2 with either mature or proDer p 1 were
     expressed in Escherichia coli or Pichia pastoris.  Forms with mutation in
     Der p 1 catalytic site were also engineered.  Purified chimeras were
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characterized by immunoblotting, circular dichroism, disulfide bond mapping, basophil and T lymphocyte stimulation assays. Results: Four fusion proteins were expressed in **E. coli** as inclusion bodies, whereas only chimeras comprising proDer p 1 were obtained in yeast. All such hybrids formed polymers and aggregates, and yeast-expressed chimeras were unstable. Circular dichroism analysis performed after refolding of bacteria expressed chimeras encompassing mature Der p 1 confirmed partial folding, consistent with the occurrence of both correct and inappropriate intramolecular disulfide bonds. All fusion molecules were recognized by Der p 1- and Der p 2-specific human IgEs, monoclonal and polyclonal antibodies. Fusion proteins activate basophils from mite-allergic patients and trigger the proliferation of specific CD4+ T cells, albeit to a lower level when compared to individual allergens. Conclusions: Production of multiple Der p 1- Der p 2 fusion proteins exhibiting partial folding and proper antigenic properties has been achieved. Nonetheless, significant solubility and stability issues currently limit the application of such chimeras for immunotherapy or diagnostic. Copyright (C) 2010 S. Karger AG, Basel

L3 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2010 ACS on STN

2009:538492 Document No. 150:512639 A Combination Vaccine for Allergy and Rhinovirus Infections Based on Rhinovirus-Derived Surface Protein VP1 and a Nonallergenic Peptide of the Major Timothy Grass Pollen Allergen Phl p 1. Edlmayr, Johanna; Niespodziana, Katarzyna; Linhart, Birgit; Focke-Tejkl, Margarete; Westritschnig, Kerstin; Scheiblhofer, Sandra; Stoecklinger, Angelika; Kneidinger, Michael; Valent, Peter; Campana, Raffaela; Thalhamer, Josef; Popow-Kraupp, Theresia; Valenta, Rudolf (Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Department of Internal Medicine I, Division of Hematology and Hemostaseology, Department of Virology, Medical University of Vienna, Vienna, 1090, Austria). Journal of Immunology, 182(10), 6298-6306 (English) 2009. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB Allergens and rhinovirus infections are among the most common elicitors of respiratory diseases. The authors report the construction of a recombinant combination vaccine for allergy and rhinovirus infections based on rhinovirus-derived VP1, the surface protein which is critically involved in infection of respiratory cells, and a non-allergenic peptide of the major grass pollen allergen Phl p 1. Recombinant hybrid mols. consisting of VP1 and a Phl p 1-derived peptide of 31 aa were expressed in **E. coli**. The hybrid mols. did not react with IgE Abs from grass pollen allergic patients and lacked allergenic activity when exposed to basophils from allergic patients. Upon immunization of mice and rabbits, the hybrids did not sensitize against Phl p 1 but induced protective IgG Abs that cross-reacted with group 1 allergens from different grass species and blocked allergic patients' IgE reactivity to Phl p 1 as well as Phl p 1-induced basophil degranulation. Moreover, hybrid-induced IgG Abs inhibited rhinovirus infection of cultured human epithelial cells. The principle of fusing non-allergenic allergen-derived peptides onto viral carrier proteins may be used for the engineering of safe **allergy vaccines** which also protect against viral infections.

L3 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2010 ACS on STN

2007:1320549 Document No. 148:142264 A hypoallergenic vaccine obtained by tail-to-head restructuring of timothy grass pollen profilin, Phl p 12, for the treatment of cross-sensitization to profilin. Westritschnig, Kerstin; Linhart, Birgit; Focke-Tejkl, Margarete; Pavkov, Tea; Keller, Walter; Ball, Tanja; Mari, Adriano; Hartl, Arnulf; Stoecklinger, Angelika; Scheiblhofer, Sandra; Thalhamer, Josef; Ferreira, Fatima; Vieths, Stefan; Vogel, Lothar; Boehm, Alexandra; Valent, Peter; Valenta, Rudolf (Christian Doppler Laboratory for Allergy Research, Division of Immunopathology,

Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria). Journal of Immunology, 179(11), 7624-7634 (English) 2007. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB Profilins are highly cross-reactive allergens in pollens and plant food. In a paradigmatic approach, the cDNA coding for timothy grass pollen profilin, Phl p 12, was used as a template to develop a new strategy for engineering an **allergy vaccine** with low IgE reactivity. Non-IgE-reactive fragments of Phl p 12 were identified by synthetic peptide chemical and restructured (rs) as a new mol., Phl p 12-rs. It comprised the C terminus of Phl p 12 at its N terminus and the Phl p 12 N terminus at its C terminus. Phl p 12-rs was expressed in **E. coli** and purified to homogeneity. Determination of secondary structure by CD indicated that the restructuring process had reduced the IgE-reactive α-helical contents of the protein but retained its β-sheet conformation. Phl p 12-rs exhibited reduced IgE binding capacity and allergenic activity but preserved T cell reactivity in allergic patients. IgG Abs induced by immunization of mice and rabbits with Phl p 12-rs cross-reacted with pollen and food-derived profilins. Recombinant Phl p 12-rs, rPhl p 12-rs, induced less reaginic IgE to the wild-type allergen than rPhl p 12. However, the rPhl p 12-rs-induced IgGs inhibited allergic patients' IgE Ab binding to profilins to a similar degree as those induced by immunization with the wild type. Phl p 12-rs specific IgG inhibited profilin-induced basophil degranulation. Thus, a restructured recombinant vaccine was developed for the treatment of profilin-allergic patients. The strategy of tail-to-head reassembly of hypoallergenic allergen fragments within one mol. represents a generally applicable strategy for the generation of **allergy vaccines**.

L3 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2010 ACS on STN

2006:498138 Document No. 145:97501 Protein and cDNA sequence of tartary buckwheat allergenic storage protein (TBc) and uses in treating allergy to Fagopyrum. Wang, Zhuanhua; Zhang, Zheng; Li, Yuying; Jing, Wei (Shanxi University, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1715410 A 20060104, 13 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2010-92438 20041224.

AB Described are the protein and cDNA sequences of tartary buckwheat allergenic storage protein (TBc) and their uses in treating allergy to Fagopyrum. The full-length cDNA sequence encoding for TBc with 515 amino acids is 1548 bp. TBc has a mol. weight of 58 kDa and contains a signal peptide sequence of 22 amino acids and a mature peptide sequence of 493 amino acids. A functional protein can be obtained by expressing an expression vector containing 3' end sequence of TBc gene in **E. coli**, and this functional protein has a mol. weight of 22kDa. Both the tartary buckwheat allergenic storage protein and said functional protein are main allergens in Fagopyrum tataricum for inducing I-type allergy reaction mediated by IgE. The invention is useful in preparing DNA vaccine and drugs for diagnosis and treatment of allergy specific to tartary buckwheat.

L3 ANSWER 5 OF 5 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN

1998:154044 Document No.: PREV199800154044. Development of BCG based **allergy vaccines**: A shuttle plasmid vector that allows production and secretion of antigens. Kumar, M. [Reprint author]; Behera, A. K.; Matsuse, H.; Lockey, R. F.; Mohapatra, S. S.. Div. Allergy and Immunol., Univ. South Florida, Tampa, FL, USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S79. print. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. Washington, DC, USA. March 13-18, 1998. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

=> s vaccine carrier
L4 1455 VACCINE CARRIER

=> s l4 and E coli
L5 53 L4 AND E COLI

=> s l5 and dead
L6 0 L5 AND DEAD

=> s l5 and killed
L7 1 L5 AND KILLED

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L7 ANSWER 1 OF 1 MEDLINE on STN
1995066342. PubMed ID: 7975861. Influence of strain viability and antigen dose on the use of attenuated mutants of Salmonella as **vaccine carriers**. Cardenas L; Dasgupta U; Clements J D. (Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.) Vaccine, (1994 Jul) Vol. 12, No. 9, pp. 833-40. Journal code: 8406899. ISSN: 0264-410X. L-ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB It is now accepted that oral **killed** typhoid vaccines are not effective at inducing protective anti-typhoid immunity. It is not known whether oral **killed** Salmonella can function as an effective carrier of other antigens to the immune system. In order to test this hypothesis, we immunized groups of mice with viable and non-viable preparations of aroA Salmonella dublin strain EL23 which codes for production of the binding subunit of the heat-labile enterotoxin of Escherichia coli (LT-B). Animals immunized orally with viable EL23 developed serum and mucosal anti-LT-B responses consistent with our previous findings. Significantly, mice immunized orally with ultraviolet-**killed** EL23 developed serum and mucosal antibody responses equivalent to those which developed in animals orally immunized with the same number of viable EL23. We extended these observations to include a number of methods of killing the organisms which may also preserve the ability of these strains to function as carriers. Our findings indicate that viability is not a requirement for use of a Salmonella strain as an immunological carrier. Moreover, our evidence indicates that bacteraemia and persistence in tissues are not necessary for oral priming, and therefore it may be best to dissociate the question of what makes the best live oral anti-typhoid vaccine from the question of what makes a good carrier of heterologous antigens.

=> s l5 and allergen
L8 0 L5 AND ALLERGEN

=> s peanut allergen
L9 1631 PEANUT ALLERGEN

=> s l9 and "Ara h6"
L10 14 L9 AND "ARA H6"

=> s l10 and E coli
L11 1 L10 AND E COLI

=> d l11 cbib abs

L11 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:320970 Document No. 135:225750 Four novel recombinant **peanut**

allergens: more information, more problems. Becker, W. -M.; Kleber-Janke, T.; Lepp, U. (Forschungszentrum Borstel, Borstel, D-23845, Germany). International Archives of Allergy and Immunology, 124(1-3), 100-102 (English) 2001. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.

AB An ideal method to clone the allergenic entities completely in peanuts is the phage display system where patients' IgE is the selection and enrichment agent. In this system, the presented allergen and its gene-containing phages are selected by patients' IgE using the panning method. The selected phages are multiplied in Escherichia coli and in the next cycle selected and enriched and so on. After five cycles, six different allergens were cloned. Two of them were the well-known major allergens of peanut, Ara h 1 and Ara h 2. Ara h 4, Ara h 5 (profilin), Ara h 6 and Ara h 7 are first described in their structure deduced from the DNA sequence. Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to seed storage proteins, whereby Ara h 6 and 7 belong to the conglutin family. Ara h 3 is an isoform of Ara h 4 with 91% identity. An elegant way to overcome the expression problems of recombinant **peanut allergens** in **E. coli** was found. This opened the way to examining the question whether certain **peanut allergens** are associated with clin. symptoms and the severity of the clin. reactions. The fact that Ara h 6 was detected by the IgE of patients with shock symptoms and urticaria but not by the IgE of patients with an isolated oral allergy syndrome may be an indication that Ara h 6 is a candidate for association with severe clin. reactions.

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L12 7 DUP REMOVE L10 (7 DUPLICATES REMOVED)

=> d l12 1-7 cbib abs

L12 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2010 ACS on STN
2010:703419 Bioinformatics comparison of **peanut allergen** Ara h2 and **Ara h6**. Xia, Li-xin; Yan, Hao; Tang, Mu-jin; Zhu, Hai; Liu, Zhi-gang (College of Medicine State Key Laboratory of Respiratory Disease for Allergy, Shenzhen University, Shenzhen, 518060, Peop. Rep. China). Shenzhen Daxue Xuebao, Ligongban, 27(2), 241-245 (Chinese) 2010. CODEN: SDXLEX. ISSN: 1000-2618. Publisher: Shenzhen Daxue Xuebao, Bianjibu.

AB The allergens Ara h2 and **Ara h6** are the most clin. relevant allergens of peanut allergies. Ara h2 can completely inhibit the IgE binding ability of **Ara h6** while **Ara h6** can only partially inhibit IgE epitope of Ara h2. Comparison between the primary and tertiary structures of Ara h2 and **Ara h6** is carried out for the exploration of this mechanism. Ara h2 contains a unique fragment (from 60 to 73) which includes two of the three major linear IgE epitopes of Ara h2. A 3-D structure of Ara h2 is obtained by homol. modeling with **Ara h6** as the template. When the structure of Ara h2 and **Ara h6** are superposed, an extra outstretched anti-parallel β-sheet linked a loop (from 58 to 72) is found within the structure of Ara h2. It also contains the sequence encoding the above-mentioned two IgE epitopes. This study gives a explanation for the difference of Ara h2 and **Ara h6** by comparison of primary and tertiary structures of Ara h2 and **Ara h6**. The explanation lays down the foundation for understanding of the mechanisms of peanut allergies and future development of hypoallergic vaccines.

L12 ANSWER 2 OF 7 EMBASE COPYRIGHT (c) 2010 Elsevier B.V. All rights reserved on STN

2009159428 EMBASE Validation of gel-free, label-free quantitative proteomics approaches: Applications for seed allergen profiling.
Stevenson, Severin E.; Thelen, Jay J. (correspondence). Department of Biochemistry, Interdisciplinary Plant Group, 109 Christopher S. Bond Life Science Center, Columbia, MO 65211, United States. thelenj@missouri.edu.
Chu, Ye; Ozias-Akins, Peggy. Department of Horticulture, University of Georgia, Tifton, GA 31793, United States.
Journal of Proteomics Vol. 72, No. 3, pp. 555-566 13 Apr 2009.
Refs: 41.
ISSN: 1874-3919.
Elsevier, P.O. Box 211, Amsterdam, 1000 AE, Netherlands.
S 1874-3919(08)00186-3. Pub. Country: Netherlands. Language: English.
Summary Language: English.
Entered STN: 20090417. Last Updated on STN: 20090417

AB Plant seeds provide a significant portion of the protein present in the human diet, but are also the major contributors of allergenic proteins that cause a majority of the reported cases of food-induced anaphylaxis. New varieties of grains and nuts as well as other seeds could be screened for allergen content before they are introduced as cultivars for food production using mass spectrometry-based quantitation approaches. Here, we present a practical comparison of gel-free and label-free methods, peak integration and spectral counting, using a linear trap mass spectrometer. The results show that both methods are linear and reproducible with protein standards from 5-200 ng, however, bioinformatic analysis for spectral counting is much simpler and therefore more amenable to high-throughput sample processing. We therefore applied spectral counting towards the analysis of transgenic peanut lines targeting the reduction of a prominent allergen. Spectral count analysis of an Ara h 2 (conglutin-7) RNA-silenced line confirmed reduction of this allergen as well as Ara h 6 (conglutin), which was further confirmed by quantitative immunoblotting. Other collateral changes include an increase in Ara h 10 (oleosin 1) in one of the three lines, a decrease in conarachin as well as increased 13-lipoxygenase and Ahy-3 (arachin) in two of three lines. .COPYRGT. 2008 Elsevier B.V.

L12 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1
2007431082. PubMed ID: 17651153. Children with peanut allergy recognize predominantly Ara h2 and **Ara h6**, which remains stable over time. Flinterman A E; van Hoffen E; den Hartog Jager C F; Koppelman S; Pasmans S G; Hoekstra M O; Bruijnzeel-Koomen C A; Knulst A C; Knol E F. (Department of Dermatology/Allergology, University Medical Centre Utrecht, The Netherlands.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2007 Aug) Vol. 37, No. 8, pp. 1221-8. Journal code: 8906443. ISSN: 0954-7894. L-ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: In peanut-allergic adults, IgE is mainly directed to Ara h1 and Ara h2. More recently, a role for **Ara h6** has been suggested. In contrast to adults, IgE in children can fluctuate over time. Therefore, children may have a more dynamic reactivity to peanut. OBJECTIVE: To examine the IgE reactivity to major **peanut allergens** in peanut-allergic children at two subsequent time-points. METHODS: Twenty children (3-15 years old) with peanut allergy, confirmed by a double-blind placebo-controlled food challenge (DBPCFC), were included. Just before and 20 months after DBPCFC, IgE reactivity to purified Ara h1, Ara h2, Ara h3 and **Ara h6** was studied by immunoblots and skin prick tests (SPTs). RESULTS: Before DBPCFC, all peanut-allergic children showed IgE reactivity to Ara h2; **Ara h6** was recognized by 16 children, and Ara h1 and Ara h3 by 10 children. After 20 months, peanut-specific IgE levels (median 23 kU/L) and the individual recognition of major allergens were comparable with the levels and recognition before challenge (median 28.2 kU/L). SPT with Ara h2 and **Ara h6** was positive in most children,

whereas SPT with Ara h1 and Ara h3 was positive in approximately half of the children. **Ara h6** induced the largest weals. None of the parameters were related to the severity of peanut allergy. CONCLUSION: Ara h2 and **Ara h6** are the most frequently recognized major **peanut allergens** in children. The individual reactivity to the major **peanut allergens** remained stable over time, despite DBPCFC.

L12 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen Ara h2 and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.
AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein production Alternatively, peanut plants are transformed with **peanut allergen** antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the Ara h2 gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein production in peanut plants. A full length genomic clone for allergen Ara h2 is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are observed A promoter region was revealed containing a putative TATA box located at position -72. Homologous regions were identified between Ara h2, h6, and h7, and between Ara h3 and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all **peanut allergen** genes and for down-regulation and silencing of multiple **peanut allergen** genes.

L12 ANSWER 5 OF 7 SCISEARCH COPYRIGHT (c) 2010 The Thomson Corporation on STN
2001:328312 The Genuine Article (R) Number: 421HV. A strategy for the identification of proteins targeted by thioredoxin.
Buchanan B B (Reprint). Univ Calif Berkeley, Dept Plant & Microbial Biol, 111 Koshland Hall, Berkeley, CA 94720 USA (Reprint). Yano H; Wong J H; Lee Y M; Cho M J. Univ Calif Berkeley, Dept Plant & Microbial Biol, Berkeley, CA 94720 USA; Univ Calif Davis, Mol Struct Facil, Davis, CA 95616 USA; Hokuriku Natl Agr Expt Stn, Niigata 9430193, Japan.
PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (10 APR 2001) Vol. 98, No. 8, pp. 4794-4799. ISSN: 0027-8424.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Thioredoxins are 12-kDa proteins functional in the regulation of cellular processes throughout the animal, plant, and microbial kingdoms. Growing evidence with seeds suggests that an h type of thioredoxin,

reduced by NADPH via NADP-thioredoxin reductase, reduces disulfide bonds of target proteins and thereby acts as a wakeup call in germination. A better understanding of the role of thioredoxin in seeds as well as other systems could be achieved if more were known about the target proteins. To this end, we have devised a strategy for the comprehensive identification of proteins targeted by thioredoxin. Tissue extracts incubated with reduced thioredoxin are treated with a fluorescent probe (monobromobimane) to label sulfhydryl groups. The newly labeled proteins are isolated by conventional two-dimensional electrophoresis: (i) nonreducing/reducing or (ii) isoelectric focusing/reducing SDS/PAGE. The isolated proteins are identified by amino acid sequencing. Each electrophoresis system offers an advantage: the first method reveals the specificity of thioredoxin in the reduction of intramolecular vs. intermolecular disulfide bonds, whereas the second method improves the separation of the labeled proteins. By application of both methods to peanut seed extracts, we isolated at least 20 thioredoxin targets and identified E-three allergens (Ara h2, Ara h3, and **Ara h6**) and two proteins not known to occur in peanut (desiccation-related and seed maturation protein). These findings open the door to the identification of proteins targeted by thioredoxin in a wide range of systems, thereby enhancing our understanding of its function and extending its technological and medical applications.

L12 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:320970 Document No. 135:225750 Four novel recombinant **peanut allergens**: more information, more problems. Becker, W. -M.; Kleber-Janke, T.; Lepp, U. (Forschungszentrum Borstel, Borstel, D-23845, Germany). International Archives of Allergy and Immunology, 124(1-3), 100-102 (English) 2001. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.

AB An ideal method to clone the allergenic entities completely in peanuts is the phage display system where patients' IgE is the selection and enrichment agent. In this system, the presented allergen and its gene-containing phages are selected by patients' IgE using the panning method. The selected phages are multiplied in Escherichia coli and in the next cycle selected and enriched and so on. After five cycles, six different allergens were cloned. Two of them were the well-known major allergens of peanut, Ara h 1 and Ara h 2. Ara h 4, Ara h 5 (profilin), Ara h 6 and Ara h 7 are first described in their structure deduced from the DNA sequence. Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to seed storage proteins, whereby Ara h 6 and 7 belong to the conglutin family. Ara h 3 is an isoform of Ara h 4 with 91% identity. An elegant way to overcome the expression problems of recombinant **peanut allergens** in E. coli was found. This opened the way to examining the question whether certain **peanut allergens** are associated with clin. symptoms and the severity of the clin. reactions. The fact that Ara h 6 was detected by the IgE of patients with shock symptoms and urticaria but not by the IgE of patients with an isolated oral allergy syndrome may be an indication that Ara h 6 is a candidate for association with severe clin. reactions.

L12 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 2
1999406463. PubMed ID: 10474031. Selective cloning of **peanut allergens**, including profilin and 2S albumins, by phage display technology. Kleber-Janke T; Crameri R; Appenzeller U; Schlaak M; Becker W M. (Research Center Borstel, Germany.. tkleber@fz-borstel.de) . International archives of allergy and immunology, (1999 Aug) Vol. 119, No. 4, pp. 265-74. Journal code: 9211652. ISSN: 1018-2438. L-ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Peanut kernels contain many allergens able to elicit IgE-mediated type 1 allergic reactions in sensitized individuals. Sera from sensitized patients recognize variable patterns of IgE-binding

proteins. The identification of the IgE-binding proteins of peanut extract would faciliate improvement of diagnostic and immunotherapeutic approaches as well as development of sensitive test systems for the detection of hidden **peanut allergens** present as additives in various industrial food products and the investigation of their stability during processing of food products. METHODS: We applied the pJuFo cloning system based on the phage surface display of functional cDNA expression products to clone cDNAs encoding **peanut allergens**. Sera (n = 40) of peanut-allergic individuals were selected according to case history, radioallergosorbent test and immunoblot analysis to demonstrate IgE binding towards the newly identified recombinant allergens. RESULTS: In addition to the known allergens Ara h 1 and Ara h 2 we were able to identify four allergens with estimated molecular weights of 36, 16, 14.5 and 14 kDa. Three of them formally termed Ara h 4, **Ara h6** and Ara h 7 show significant sequence similarities to the family of seed storage proteins and the fourth (Ara h 5) corresponds to the well-known plant allergen profilin. Immunoblotting of the six expressed recombinant allergens with 40 patients sera shows 14 individual recognition patterns and the following frequency of specific IgE binding: Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% of the selected sera. CONCLUSIONS: All of the selected peanut-positive sera can detect at least one of the six identified recombinant allergens which can be used to establish individual patients' reactivity profiles. A comparison of these profiles with the clinical data will possibly allow a further insight into the relationship between clinical severity of the symptoms and specific IgE levels towards the six **peanut allergens**.

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=> s l9 and recombinant
L13        195 L9 AND RECOMBINANT

=> s l13 and conglutin
L14          7 L13 AND CONGLUTIN

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15          3 DUP REMOVE L14 (4 DUPLICATES REMOVED)

=> d l15 1-3 cbib abs

L15  ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2010 ACS on STN
2007:1052514   Document No. 147:384048  Modified vaccinia virus Ankara (MVA)
     encoding a recombinant allergen for the treatment of type I
     hypersensitivity in animals and humans.  Albrecht, Melanie; Sutter, Gerd;
     Suezer, Yasemin; Reese, Gerald; Vieths, Stefan; Staib, Caroline
     (Paul-Ehrlich-Institut Bundesamt fuer Sera und Impfstoffe, Germany).  Eur.
     Pat. Appl. EP 1835031 A1 20070919, 40pp.  DESIGNATED STATES: R:  AT, BE,
     BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LT,
     LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, AL, BA, HR, MK, YU.
     (English).  CODEN: EPXXDW.  APPLICATION: EP 2006-5164 20060314.
AB   The present invention relates to the use of a recombinant
     modified vaccinia virus Ankara (MVA) comprising a heterologous nucleic
     acid for the production of a medicament for the prevention and/or treatment of
     type I hypersensitivity in a living animal including humans.  The
     invention further relates to a recombinant modified vaccinia
     virus Ankara (MVA) comprising a heterologous nucleic acid, wherein the
     heterologous nucleic acid is incorporated into a non-essential region of
     the genome of the MVA, the heterologous nucleic acid is under the control
     of, e.g. a vaccinia virus-specific promoter and, the heterologous nucleic
     acid is selected from the group of nucleic acids encoding an allergen
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selected from the group of weed pollens, grass pollens, tree pollens, mites, animals, fungi, insects, rubber, worms, human autoallergens, and foods.

L15 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
2003475502. PubMed ID: 14550644. High-yield expression in Escherichia coli, purification, and characterization of properly folded major **peanut allergen** Ara h 2. Lehmann Katrin; Hoffmann Silke; Neudecker Philipp; Suhr Martin; Becker Wolf-Meinhard; Rosch Paul. (Lehrstuhl Biopolymere, Universitat Bayreuth 30, Universitaetsstrasse 30, 95440, Bayreuth, Germany.) Protein expression and purification, (2003 Oct) Vol. 31, No. 2, pp. 250-9. Journal code: 9101496. ISSN: 1046-5928. L-ISSN: 1046-5928. Pub. country: United States. Language: English.
AB Allergic reactions to peanuts are a serious health problem because of their high prevalence, associated with potential severity, and chronicity. One of the three major allergens in peanut, Ara h 2, is a member of the **conglutin** family of seed storage proteins. Ara h 2 shows high sequence homology to proteins of the 2S albumin family. Presently, only very few structural data from allergenic proteins of this family exist. For a detailed understanding of the molecular mechanisms of food-induced allergies and for the development of therapeutic strategies knowledge of the high-resolution three-dimensional structure of allergenic proteins is essential. We report a method for the efficient large-scale preparation of properly folded Ara h 2 for structural studies and report CD-spectroscopic data. In contrast to other allergenic 2S albumins, Ara h 2 exists as a single continuous polypeptide chain in peanut seeds, and thus heterologous expression in Escherichia coli was possible. Ara h 2 was expressed as Trx-His-tag fusion protein in E. coli Origami (DE3), a modified E. coli strain with oxidizing cytoplasm which allows the formation of disulfide bridges. It could be shown that **recombinant** Ara h 2, thus overexpressed and purified, and the allergen isolated from peanuts are identical as judged from immunoblotting, analytical HPLC, and circular dichroism spectra.

L15 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:320970 Document No. 135:225750 Four novel **recombinant peanut allergens**: more information, more problems. Becker, W. -M.; Kleber-Janke, T.; Lepp, U. (Forschungszentrum Borstel, Borstel, D-23845, Germany). International Archives of Allergy and Immunology, 124(1-3), 100-102 (English) 2001. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.
AB An ideal method to clone the allergenic entities completely in peanuts is the phage display system where patients' IgE is the selection and enrichment agent. In this system, the presented allergen and its gene-containing phages are selected by patients' IgE using the panning method. The selected phages are multiplied in Escherichia coli and in the next cycle selected and enriched and so on. After five cycles, six different allergens were cloned. Two of them were the well-known major allergens of peanut, Ara h 1 and Ara h 2. Ara h 4, Ara h 5 (profilin), Ara h 6 and Ara h 7 are first described in their structure deduced from the DNA sequence. Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to seed storage proteins, whereby Ara h 6 and 7 belong to the **conglutin** family. Ara h 3 is an isoform of Ara h 4 with 91% identity. An elegant way to overcome the expression problems of **recombinant peanut allergens** in E. coli was found. This opened the way to examining the question whether certain **peanut allergens** are associated with clin. symptoms and the severity of the clin. reactions. The fact that Ara h 6 was detected by the IgE of patients with shock symptoms and urticaria but not by the IgE of patients with an isolated oral allergy syndrome may be an indication that Ara h 6 is a candidate for association with severe clin. reactions.

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=> s E coli
L16     512192 E COLI

=> s l16 and recombinant allergen
L17        242 L16 AND RECOMBINANT ALLERGEN

=> s l17 and pd<20000406
L18        104 L17 AND PD<20000406

=> s l8 and modified allergen
L19          0 L8 AND MODIFIED ALLERGEN

=> s l18 and reduced IgE
L20          0 L18 AND REDUCED IGE

=> s l18 and IgE
L21         89 L18 AND IGE

=> s l21 and modified
L22          1 L21 AND MODIFIED

=> d l22 cbib abs

L22  ANSWER 1 OF 1     MEDLINE on STN
2000420411.    PubMed ID: 10877820.   Rapid production of the major birch
     pollen allergen Bet v 1 in Nicotiana benthamiana plants and its
     immunological in vitro and in vivo characterization. Krebitz M; Wiedermann
     U; Essl D; Steinkellner H; Wagner B; Turpen T H; Ebner C; Scheiner O;
     Breiteneder H. (Department of Pathophysiology, University of Vienna,
     Austria. ) The FASEB journal : official publication of the Federation of
     American Societies for Experimental Biology, (2000 Jul) Vol. 14,
     No. 10, pp. 1279-88.  Journal code: 8804484. ISSN: 0892-6638. L-ISSN:
     0892-6638. Pub. country: United States. Language: English.
AB   Type I allergies are immunological disorders that afflict a quarter of the
     world's population.  Improved diagnosis of allergic diseases and the
     formulation of new therapeutic approaches are based on the use of
     recombinant allergens.  We describe here for the first
     time the application of a rapid plant-based expression system for a
     plant-derived allergen and its immunological characterization.  We
     expressed our model allergen Bet v 1, the major birch pollen allergen, in
     the tobacco-related species Nicotiana benthamiana using a tobacco mosaic
     virus vector.  Two weeks postinoculation, plants infected with recombinant
     viral RNA containing the Bet v 1 coding sequence accumulated the allergen
     to levels of 200 microg/g leaf material.  Total nonpurified protein
     extracts from plants were used for immunological characterizations.
     IgE immunoblots and ELISA (enzyme-linked immunoassay) inhibition
     assays showed comparable IgE binding properties for tobacco
     recombinant (r) Bet v 1 and natural (n) Bet v 1, suggesting that the B
     cell epitopes were preserved when the allergen was expressed in N.
     benthamiana plants.  Using a murine model of type I allergy, mice
     immunized with crude leaf extracts containing Bet v 1 with purified rBet v
     1 produced in E. coli or with birch pollen extract
     generated comparable allergen-specific IgE and IgG1 antibody
     responses and positive type I skin test reactions.  These results
     demonstrate that nonpurified Bet v 1 overexpressed in N. benthamina has
     the same immunogenicity as purified Bet v 1 produced in E.
     coli or nBet v 1.  We therefore conclude that this plant
     expression system offers a viable alternative to fermentation-based
     production of allergens in bacteria or yeasts.  In addition, there may be
     a broad utility of this system for the development of new and low-cost
     vaccination strategies against allergy.
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=> s microbial delivery
L23 47 MICROBIAL DELIVERY

=> s l23 and peanut
L24 1 L23 AND PEANUT

=> d l24 cbib abs

L24 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:676622 Document No. 135:225857 **Microbial delivery** system. Caplan, Michael (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.
AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to allergens or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce allergens and protect the subjects from exposure to the allergens until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. bacteria, gram-pos. bacteria, and yeast. Particularly preferred allergens are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

=> s l23 and allergen
L25 1 L23 AND ALLERGEN

=> d l25 cbib abs

L25 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2010 ACS on STN
2001:676622 Document No. 135:225857 **Microbial delivery** system. Caplan, Michael (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2001066136 A2 20010913, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33121 20001206. PRIORITY: US 2000-PV195035 20000306.
AB The present invention provides methods and compns. for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to **allergens** or susceptible to allergies. Methods of the present invention utilize

administration of microorganisms to subjects, where the microorganisms produce **allergens** and protect the subjects from exposure to the **allergens** until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-neg. bacteria, gram-pos. bacteria, and yeast. Particularly preferred **allergens** are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

=> s (caplan m?/au or sampson h?/au or burks A?/au or burks w?/au or bottomly k?/au or sosin h?/au)
L26 6338 (CAPLAN M?/AU OR SAMPSON H?/AU OR BURKS A?/AU OR BURKS W?/AU OR BOTTOMLY K?/AU OR SOSIN H?/AU)

=> s l26 and E coli
L27 28 L26 AND E COLI

=> s l27 and allergen
L28 10 L27 AND ALLERGEN

=> dup remove l28
PROCESSING COMPLETED FOR L28
L29 5 DUP REMOVE L28 (5 DUPLICATES REMOVED)

=> d l29 1-5 cbib abs

L29 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2010 ACS on STN
2005:259357 Document No. 142:334946 Recombinant **allergens** with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex **allergens**. **Caplan, Michael J.; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A.** (USA). U.S. Pat. Appl. Publ. US 20050063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.
AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein **allergen**. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein **allergen**. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as **E. coli** . Any **allergen** may be used in the inventive methods. Particularly preferred **allergens** are anaphylactic **allergens** including protein **allergens** found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L29 ANSWER 2 OF 5 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN
2005:536746 Document No.: PREV200510331749. Peanut **allergens** and methods. **Burks,, A. Wesley** [Inventor]; Stanley, J. Steven [Inventor]; Bannon, Gary A. [Inventor]; Cockrell, Gael [Inventor]; Helm, Ricki M. [Inventor]. Little Rock, AR USA. ASSIGNEE: University of Arkansas. Patent Info.: US 06835824 20041228. Official Gazette of the United States Patent and Trademark Office Patents, (DEC 28 2004) CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB One of the major peanut **allergens**, Ara h I, was selected from cDNA expression library clones using Ara h I specific oligo-nucleotides and polymerase chain reaction technology. The Ara h I clone identified a 2.3 kb mRNA species on a Northern blot containing peanut poly A+RNA. DNA sequence analysis of the cloned inserts revealed that the Ara h I **allergen** has significant homology with the vicilin seed storage protein family found in most higher plants. The isolation of the Ara h I clones allowed the synthesis of this protein in **E. coli** cells and subsequent recognition of this. recombinant protein in immunoblot analysis using serum IgE from patients with peanut hypersensitivity.

L29 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2010 ACS on STN DUPLICATE 1
2004:1121739 Document No. 142:278913 Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M.; **Burks, A. Wesley** (Department of Microbiology/Immunology, University of Arkansas for Medical Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.
AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with heat-killed **E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

L29 ANSWER 4 OF 5 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN
1999:134462 Document No.: PREV199900134462. Modulation of the allergenicity of a major peanut **allergen**, Ara h 2 by mutagenesis of its immunodominant IgE binding epitopes. King, N. [Reprint author]; Maleki, S. J.; **Sampson, H.; Burks, A. W.** [Reprint author]; Bannon, G. A. [Reprint author]. Univ. Arkansas Med. Sci., Little Rock, AR 72201, USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S67. print.
Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. Orlando, Florida, USA. February 26-March 3, 1999. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L29 ANSWER 5 OF 5 MEDLINE on STN DUPLICATE 2
1996013631. PubMed ID: 7560062. Recombinant peanut **allergen** Ara h I expression and IgE binding in patients with peanut hypersensitivity. **Burks A W**; Cockrell G; Stanley J S; Helm R M; Bannon G A. (Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock 72205, USA.) The Journal of clinical investigation, (1995 Oct) Vol. 96, No. 4, pp. 1715-21. Journal code: 7802877. ISSN: 0021-9738. L-ISSN: 0021-9738.
Report No.: NLM-PMC185807. Pub. country: United States. Language: English.

AB Peanut allergy is a significant health problem because of the frequency, the potential severity, and the chronicity of the allergic sensitivity. Serum IgE from patients with documented peanut hypersensitivity reactions and a peanut cDNA expression library were used to identify clones that encode peanut **allergens**. One of the major peanut **allergens**, Ara h I, was selected from these clones using Ara h I specific oligonucleotides and polymerase chain reaction technology. The Ara h I clone identified a 2.3-kb mRNA species on a Northern blot containing peanut poly (A)+ RNA. DNA sequence analysis of the cloned inserts revealed that the Ara h I **allergen** has significant homology with the vicilin seed storage protein family found in most higher plants. The isolation of the Ara h I clones allowed the synthesis of this protein in **E. coli** cells and subsequent recognition of this recombinant protein in immunoblot analysis using serum IgE from patients with peanut hypersensitivity. With the production of the recombinant peanut protein it will now be possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general

=> s peanut allergen
L30 1631 PEANUT ALLERGEN

=> s l30 and "Ara h6"
L31 14 L30 AND "ARA H6"

=> s l31 and IgE epitope
L32 1 L31 AND IGE EPITOPE

=> d l32 cbib abs

L32 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2010 ACS on STN
2010:703419 Bioinformatics comparison of **peanut allergen** Ara h2 and **Ara h6**. Xia, Li-xin; Yan, Hao; Tang, Mu-jin; Zhu, Hai; Liu, Zhi-gang (College of Medicine State Key Laboratory of Respiratory Disease for Allergy, Shenzhen University, Shenzhen, 518060, Peop. Rep. China). Shenzhen Daxue Xuebao, Ligongban, 27(2), 241-245 (Chinese) 2010. CODEN: SDXLEX. ISSN: 1000-2618. Publisher: Shenzhen Daxue Xuebao, Bianjibu.

AB The allergens Ara h2 and **Ara h6** are the most clin. relevant allergens of peanut allergies. Ara h2 can completely inhibit the IgE binding ability of **Ara h6** while **Ara h6** can only partially inhibit **IgE epitope** of Ara h2. Comparison between the primary and tertiary structures of Ara h2 and **Ara h6** is carried out for the exploration of this mechanism. Ara h2 contains a unique fragment (from 60 to 73) which includes two of the three major linear **IgE epitopes** of Ara h2. A 3-D structure of Ara h2 is obtained by homol. modeling with **Ara h6** as the template. When the structure of Ara h2 and **Ara h6** are superposed, an extra outstretched anti-parallel β-sheet linked a loop (from 58 to 72) is found within the structure of Ara h2. It also contains the sequence encoding the above-mentioned two **IgE epitopes**. This study gives a explanation for the difference of Ara h2 and **Ara h6** by comparison of primary and tertiary structures of Ara h2 and **Ara h6**. The explanation lays down the foundation for understanding of the mechanisms of peanut allergies and future development of hypoallergic vaccines.

=> s heat killed E coli

L33 323 HEAT KILLED E COLI

=> s l33 and allergy
L34 10 L33 AND ALLERGY

=> dup remove l34
PROCESSING COMPLETED FOR L34
L35 3 DUP REMOVE L34 (7 DUPLICATES REMOVED)

=> d l35 1-3 cbib abs

L35 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
2009745033. PubMed ID: 19833393. Attenuation of **allergy** to ovomucoid in pigs by neonatal treatment with heat-killed Escherichia coli or E. coli producing porcine IFN-gamma. Rupa Prithy; Schmied Julie; Lai Serene; Wilkie Bruce N. (Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.) Veterinary immunology and immunopathology, (2009 Nov 15) Vol. 132, No. 1, pp. 78-83. Electronic Publication: 2009-09-24. Journal code: 8002006. E-ISSN: 1873-2534. L-ISSN: 0165-2427. Pub. country: Netherlands. Language: English.

AB Food **allergy** is epidemic and prompts investigation to reduce allergic predisposition. It was hypothesized that heat-killed Escherichia coli injected intramuscularly (im) with or without interferon gamma (IFN-gamma), reduces neonatal susceptibility to experimental egg **allergy**. Two litters of Yorkshire pigs were assigned to three intramuscular treatment groups (four/group): control (PBS), **heat-killed E. coli** with or without IFN-gamma-expressing plasmid. Pigs were sensitized to ovomucoid (Ovm) by intraperitoneal injection with cholera toxin. To assess induction of **allergy**, pigs were fed egg white in yoghurt and assigned scores for allergic signs. Significantly fewer pigs developed **allergy** and passive cutaneous anaphylaxis in E. coli and E. coli+IFN-gamma vs control groups. E. coli-treated pigs also had significantly lower frequency of mean clinical scores. E. coli and E. coli+IFN-gamma groups did not differ. Serum antibody associated with IgG (H & L), IgG(1), IgG(2) or IgE all correlated but did not differ by treatment groups. Thus, treatment of neonatal pigs with **heat-killed E. coli** by im injection reduced susceptibility to allergic sensitization with Ovm. Inclusion of the type-1 cytokine, IFN-gamma, had no additional effect. Results indicate a method for prophylaxis of **allergy** and suggest support for the "hygiene hypothesis".

L35 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2010 ACS on STN DUPLICATE 2
2004:1121739 Document No. 142:278913 Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M.; Burks, A. Wesley (Department of Microbiology/Immunology, University of Arkansas for Medical Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.

AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with **heat-killed E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was

provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food **allergies** in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

L35 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN
2004:229282 Document No.: PREV200400229798. Generation of **heat-killed E. coli** expressing cow milk proteins for immunotherapy regiment for the milk allergic mice. Grishin, A. [Reprint Author]; Srivastava, K. [Reprint Author]; Sampson, H. [Reprint Author]; Li, X. [Reprint Author]. Pediatrics, Mount Sinai School of Medicine, New York, NY, USA. Journal of Allergy and Clinical Immunology, (February 2004) Vol. 113, No. 2 Supplement, pp. S325. print.
Meeting Info.: 60th Annual Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). San Francisco, CA, USA. March 19-23, 2004. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

=> s l33 and allergen
L36 3 L33 AND ALLERGEN

=> dup remove l36
PROCESSING COMPLETED FOR L36
L37 2 DUP REMOVE L36 (1 DUPLICATE REMOVED)

=> d l37 1-2 cbib abs

L37 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN
2010:38192 Document No.: PREV201000038192. Attenuation of allergy to ovomucoid in pigs by neonatal treatment with heat-killed Escherichia coli or E-coli producing porcine IFN-gamma. Rupa, Prithy; Schmied, Julie; Lai, Serene; Wilkie, Bruce N. [Reprint Author]. Univ Guelph, Ontario Vet Coll, Dept Pathobiol, Guelph, ON N1G 2W1, Canada. bwilkie@uoguelph.ca. Veterinary Immunology and Immunopathology, (NOV 15 2009) Vol. 132, No. 1, Sp. Iss. 1, pp. 78-83.
CODEN: VIIMDS. ISSN: 0165-2427. Language: English.
AB Food allergy is epidemic and prompts investigation to reduce allergic predisposition. It was hypothesized that heat-killed Escherichia coli injected intramuscularly (im) with or without interferon gamma (IFN-gamma), reduces neonatal susceptibility to experimental egg allergy. Two litters of Yorkshire pigs were assigned to three intramuscular treatment groups (four/group): control (PBS), **heat-killed E coli** with or without IFN-gamma-expressing plasmid. Pigs were sensitized to ovomucoid (Ovm) by intraperitoneal injection with cholera toxin. To assess induction of allergy, pigs were fed egg white in yoghurt and assigned scores for allergic signs. Significantly fewer pigs developed allergy and passive cutaneous anaphylaxis in E. call and E. coli + IFN-gamma vs control groups. E. coli-treated pigs also had significantly lower frequency of mean clinical scores. E. coli and E. coli + IFN-gamma groups did not differ. Serum antibody associated with IgG (H & L), IgG(1), IgG(2) or IgE all correlated but did not differ by treatment groups. Thus, treatment of neonatal pigs with **heat-killed E coli** by im injection reduced susceptibility to allergic sensitization with Ovm. Inclusion of the type-1 cytokine, IFN-gamma, had no additional effect. Results indicate a method for prophylaxis of

allergy and suggest support for the "hygiene hypothesis". (C) 2009 Elsevier B.V. All rights reserved.

L37 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2010 ACS on STN DUPLICATE 1
2004:1121739 Document No. 142:278913 Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M.; Burks, A. Wesley (Department of Microbiology/Immunology, University of Arkansas for Medical Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.
AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with **heat-killed E. coli** prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

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L38 17 L33 AND VACCINE

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L39 16 L38 AND PD<20000406

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L41 1 L40 AND TH1

=> d l41 cbib abs

L41 ANSWER 1 OF 1 MEDLINE on STN
1996101747. PubMed ID: 7494229. Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing Escherichia coli. Fenton R G; Keller C J; Hanna N; Taub D D. (Division of Clinical Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.) Journal of the National Cancer Institute, **(1995 Dec 20)** Vol. 87, No. 24, pp. 1853-61. Journal code: 7503089. ISSN: 0027-8874. L-ISSN: 0027-8874. Pub. country: United States. Language: English.
AB BACKGROUND: Point mutations in the ras proto-oncogene that activate its oncogenic potential occur in approximately 30% of human cancers. Previous studies have demonstrated that T-cell immunity against some forms of mutant Ras proteins could be elicited, and some effectiveness against tumors expressing activated Ras has been reported. PURPOSE: The goal of this study was to determine if immunization of mice with two forms of

mutant Ras protein can induce high levels of Ras mutation-specific T-cell immunity in vitro and tumor regression in vivo. METHODS: Mice (BALB/c or C3H/HeJ) were immunized subcutaneously at 2-week intervals with purified Ras oncoproteins mixed with the immunologic adjuvants Antigen Formulation or QS-21, both of which have been shown to enhance the induction of T-cell-mediated immunity when included as components of soluble protein **vaccines**. In some experiments, mice were immunized directly with heat-killed Escherichia coli that had been induced to express one of the mutant Ras proteins. Spleen cells plus lymph node cells from Ras-immunized mice were tested in vitro for lysis of syngeneic Ras-expressing tumor cells and proliferation in response to mutant Ras peptides. For some of the cytolytic activity experiments, the spleen cells were grown under **TH1** conditions (growth in presence of interleukin 2, interferon gamma, and an antibody directed against interleukin 4 to stimulate a cell-mediated immune response) or TH2 conditions (growth in presence of interleukins 2 and 4 to stimulate a humoral immune response). The specificity of immunity was examined in vivo by challenge of Ras-immunized mice with syngeneic tumor cells expressing mutant Ras oncoproteins (HaBalb, i.e., BALB/c mouse cells expressing Ras with arginine substituted at amino acid position 12 [Arg 12 Ras]; C3HL61, i.e., C3H/HeJ mouse cells expressing Ras with leucine substituted at position 61 [Leu 61 Ras]). Ten mice per group were used in each experiment. RESULTS: Proliferative and cytolytic T-cell responses directed against the Arg 12 Ras protein were generated in BALB/c mice, resulting in protection against challenge with cells expressing Arg 12 Ras and therapeutic benefit in mice bearing established tumors expressing this protein. In C3H/HeJ mice, high levels of cytolytic and proliferative responses were induced against Leu 61 Ras. Immunization with **heat -killed E. coli** genetically engineered to express Leu 61 Ras also led to the induction of anti-Ras T-cell immunity. T cells grown under **TH1** conditions were cytolytic against Ras-transformed tumor cells, whereas those grown under TH2 conditions were not. CONCLUSIONS: Immunization as described here leads to Ras mutation-specific antitumor immunity in vitro and in vivo, with therapeutic efficacy in an established tumor model.

=> d l40 1-8 cbib abs

L40 ANSWER 1 OF 8 MEDLINE on STN DUPLICATE 1
1996101747. PubMed ID: 7494229. Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing Escherichia coli. Fenton R G; Keller C J; Hanna N; Taub D D. (Division of Clinical Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.) Journal of the National Cancer Institute, **(1995 Dec 20)** Vol. 87, No. 24, pp. 1853-61. Journal code: 7503089. ISSN: 0027-8874. L-ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Point mutations in the ras proto-oncogene that activate its oncogenic potential occur in approximately 30% of human cancers. Previous studies have demonstrated that T-cell immunity against some forms of mutant Ras proteins could be elicited, and some effectiveness against tumors expressing activated Ras has been reported. PURPOSE: The goal of this study was to determine if immunization of mice with two forms of mutant Ras protein can induce high levels of Ras mutation-specific T-cell immunity in vitro and tumor regression in vivo. METHODS: Mice (BALB/c or C3H/HeJ) were immunized subcutaneously at 2-week intervals with purified Ras oncoproteins mixed with the immunologic adjuvants Antigen Formulation or QS-21, both of which have been shown to enhance the induction of T-cell-mediated immunity when included as components of soluble protein **vaccines**. In some experiments, mice were immunized directly with heat-killed Escherichia coli that had been induced to express one of the

mutant Ras proteins. Spleen cells plus lymph node cells from Ras-immunized mice were tested in vitro for lysis of syngeneic Ras-expressing tumor cells and proliferation in response to mutant Ras peptides. For some of the cytolytic activity experiments, the spleen cells were grown under TH1 conditions (growth in presence of interleukin 2, interferon gamma, and an antibody directed against interleukin 4 to stimulate a cell-mediated immune response) or TH2 conditions (growth in presence of interleukins 2 and 4 to stimulate a humoral immune response). The specificity of immunity was examined in vivo by challenge of Ras-immunized mice with syngeneic tumor cells expressing mutant Ras oncoproteins (HaBalb, i.e., BALB/c mouse cells expressing Ras with arginine substituted at amino acid position 12 [Arg 12 Ras]; C3HL61, i.e., C3H/HeJ mouse cells expressing Ras with leucine substituted at position 61 [Leu 61 Ras]). Ten mice per group were used in each experiment. RESULTS: Proliferative and cytolytic T-cell responses directed against the Arg 12 Ras protein were generated in BALB/c mice, resulting in protection against challenge with cells expressing Arg 12 Ras and therapeutic benefit in mice bearing established tumors expressing this protein. In C3H/HeJ mice, high levels of cytolytic and proliferative responses were induced against Leu 61 Ras. Immunization with **heat-killed E. coli** genetically engineered to express Leu 61 Ras also led to the induction of anti-Ras T-cell immunity. T cells grown under TH1 conditions were cytolytic against Ras-transformed tumor cells, whereas those grown under TH2 conditions were not. CONCLUSIONS: Immunization as described here leads to Ras mutation-specific antitumor immunity in vitro and in vivo, with therapeutic efficacy in an established tumor model.

L40 ANSWER 2 OF 8 BIOSIS COPYRIGHT (c) 2010 The Thomson Corporation on STN
1985:233270 Document No.: PREV198579013266; BA79:13266. IMMUNOCONGLUTININ LEVELS IN CHICKS VACCINATED WITH SALMONELLA-GALLINARUM 9R SALMONELLA-PULLORUM E-79 OR ESCHERICHIA-COLI 020 **VACCINES** AND EXPERIMENTALLY INFECTED WITH SALMONELLA-GALLINARUM. JAISWAL T N [Reprint author]; MITTAL K R. COLLEGE OF VET SCIENCE AND ANIMAL HUSBANDRY, GUJARAT AGRIC UNIVERSITY, SK NAGAR, DANTIWADA, BANASKANTHA-395 506. Indian Veterinary Medical Journal, (**1984**) Vol. 8, No. 1, pp. 9-13. CODEN: IVMJDL. ISSN: 0250-5266. Language: ENGLISH.
AB Vaccination of chicks with live S. gallinarum (9R) **vaccines** with or without adjuvant caused an initial fall in the levels of pre-existing autostimulated immunoconglutinin (IK) by the 10th day but a slow increase in the IK level by 21st day postvaccination. Heat-killed S. pollorum (E79) and **heat-killed E. coli** (020) **vaccines** caused no such reduction in the IK level during the post-vaccination period. An increase in the IK level during post-vaccination period in these groups of chickens were observed. Challenge infection with S. gallinarium (V) in all the vaccinated groups of birds showed a marked decrease in IK level during the early challenge period indicating the involvement of IK in the host parasite reaction. The IK level increased by the 21st day post-challenge. Evidently, involvement of IK may help in host defense only in initial stages but eventually fail to protect chicks against S. gallinarum infection when the causative agent manages to enter the cells when both specific antibodies and nonspecific serum factor like IK fail to be effective.

L40 ANSWER 3 OF 8 MEDLINE on STN DUPLICATE 2
1982190380. PubMed ID: 7042755. Induction of immunity against lethal Haemophilus influenzae type b infection by Escherichia coli core lipopolysaccharide. Marks M I; Ziegler E J; Douglas H; Corbeil L B; Braude A I. The Journal of clinical investigation, **(1982 Apr)** Vol. 69, No. 4, pp. 742-9. Journal code: 7802877. ISSN: 0021-9738. L-ISSN: 0021-9738.
Report No.: NLM-PMC370127. Pub. country: United States. Language: English.
AB Efforts to prevent Haemophilus influenzae type b (HIB) infections in

infancy have been hampered by the low immunogenicity of capsular polysaccharide **vaccines** in children younger than 18 mos. In searching for alternate immunogens, we have studied the protective potential of polysaccharide-poor, lipid-rich endotoxin (LPS) core in experimental HIB infections. Because all gram-negative bacteria have similar LPS core structures, we were able to use as **vaccine** the J5 mutant of Escherichia coli 0111, the LPS of which consists only of core components, and thus to avoid problems in interpretation arising from **vaccine** contamination with non-LPS HIB immunogens. Mice were given graded inocula of HIB and developed lethal infection analogous to human HIB disease when virulence was enhanced with mucin and hemoglobin. After active immunization with **heat-killed E. coli** J5, 40/50 (80%) of infected mice survived, compared with 14/50 (28%) of saline-immunized controls (P less than 0.005). Passive immunization with rabbit antiserum against E. coli J5 prevented lethal HIB infection when administered 24 or 72 h before or 3 h after infection. This protection was abolished by adsorption of antiserum with purified J5 LPS, with survival reduced from 14/24 to 0/24 (P less than 0.005). Furthermore, rabbit antiserum to purified J5 LPS gave just as potent protection against death as antiserum to whole J5 cells. These studies demonstrate that immunity to core LPS confers protection against experimental murine HIB infection and provide the framework for a new approach to prevention of human disease from HIB.

L40 ANSWER 4 OF 8 MEDLINE on STN DUPLICATE 3

1981281536. PubMed ID: 7023456. Consequences of active or passive immunization of turkeys against Escherichia coli O78. Arp L H. Avian diseases, **(1980 Oct-Dec)** Vol. 24, No. 4, pp. 808-15. Journal code: 0370617. ISSN: 0005-2086. L-ISSN: 0005-2086. Pub. country: United States. Language: English.

AB Turkeys were injected at 7 and 14 days of age with live, heat-killed or formalin-killed Escherichia coli O78. Other turkeys were passively immunized at 22 days of age with hyperimmune serum produced against live or **heat-killed E. coli** O78. All turkeys were challenged at 24 days of age with E. coli O78. Turkeys immunized intramuscularly or intratracheally with live E. coli O78 were protected from death, whereas few turkeys given killed E. coli O78 were protected. Passively immunized turkeys were protected from death regardless of whether live or **heat-killed E. coli** O78 was used to produce the hyperimmune serum. Most turkeys that survived challenge developed septic polysynovitis 2--4 days after challenge.

L40 ANSWER 5 OF 8 MEDLINE on STN

1976189304. PubMed ID: 818014. Antiviral activity of Brucella abortus preparations; separation of active components. Feingold D S; Keleti G; Youngner J S. Infection and immunity, **(1976 Mar)** Vol. 13, No. 3, pp. 763-7. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567. Report No.: NLM-PMC420675. Pub. country: United States. Language: English.

AB Injection into mice of heat-killed Brucella abortus or aqueous ether-extracted B. abortus (Bru-pel) induced a "virus-type" interferon response, with peak titers at 6.5 h. The animals also were protected against challenge with otherwise lethal doses of Semliki forest virus. Extraction of either heated B. abortus or BRU-PEL with a mixture of chloroform-methanol (2:1, vol/vol) (C-M(yielded an insoluble residue (extracted cells) and a C-M extract. Neither extracted cells nor C-M extract alone induced interferon or afforded protection against Semliki forest virus infection in mice. Full interferon-inducing and protective activity was restored when extracted cells were recombined with C-M extract. C-M extract from heat-killed Escherichia coli also was effective in restoring activity to extracted Brucella cells. Neither **heat-killed E. coli** nor its C-M extract was

active, nor was C-M estracted E. coli recombined with the C-M extract from B. abortus. These results suggest that the interferon-inducing and antiviral protective properties of B. abortus are constituted of a C-M-extractable component that is common to B. abortus and E. coli and an unextractable component that is unique to B. abortus.

L40 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2010 ACS on STN
1974:567727 Document No. 81:167727 Original Reference No. 81:25959a,25962a Intestinal antibody secretion in the young pig in response to oral immunization with Escherichia coli. Porter, P.; Kenworthy, R.; Noakes, D. E.; Allen, W. D. (Unilever Res., Sharnbrook/Bedford, UK). Immunology, 27(5), 841-53 (English) **1974**. CODEN: IMMUAM. ISSN: 0019-2805.
AB Intestinal immunoglobulins and antibodies in the local immune response to E. coli O somatic antigens was studied in young fistulated pigs. Antibody levels in intestinal secretion were raised for .apprx.2-3 weeks following a single local antigenic challenge with a heat-killed aqueous suspension of E. coli. A 2nd challenge provoked a similar response suggesting a lack of immunol. memory. Antibody activity in the secretions was predominantly associated with IgA and immunofluorescent studies of biopsy specimens from these pigs indicated that intestinal synthesis and secretion of IgA had begun by the 10th day of life. Studies of piglets reared with the sow indicated that oral immunization with E. coli antigen after 10 days of age stimulated intestinal antibody secretion before weaning at 3 weeks. The response of gnotobiotic pigs to oral immunization and infection was evaluated by immunofluorescent histol. of the intestinal mucosa. Repeated oral administration of **heat-killed E. coli** O8 gave an immunocyte response in the lamina propria numerically comparable with that produced by infection. The early response was dominated by cells of the IgM class whereas after 3 weeks IgA cells predominated. In the germ-free animal very few immunoglobulin-containing cells were detected. In vitro studies of antibacterial activity indicated that the most probable mechanism of immunol. control in the alimentary tract is bacteriostasis.

L40 ANSWER 7 OF 8 EMBASE COPYRIGHT (c) 2010 Elsevier B.V. All rights reserved on STN
1975027648 EMBASE The effect of active immunisation on ascending pyelonephritis in the rat.
Radford, N.J.; Chick, S.; Ling, R.; et. al.. KRUF Inst. Ren. Dis., Welsh Nat. Sch. Med., Roy. Infirm., Cardiff, United Kingdom.
Journal of Pathology Vol. 112, No. 3, pp. 169-175 **1974**.
ISSN: 0022-3417. CODEN: JPBAA7. Language: English.
AB In the rat, active immunization with **heat killed E. coli** serotype 078 **vaccine** produced a high titer of IgM anti O antibody after 14 days. At this time, lower titers of IgG anti O antibodies were found in some of the animals. These antibodies did not prevent bacterial invasion of the kidney nor did they affect the incidence or severity of the renal scarring following ascending infection with E. coli serotype 078. Fourteen days after immunization with a formalin killed **vaccine** very high titers of IgM and IgG anti K antibodies were noted; these were in excess of 1 in 5120. It was shown that these antibodies reduced the severity but not the frequency of renal scarring following ascending E. coli infection.

L40 ANSWER 8 OF 8 MEDLINE on STN DUPLICATE 4
1971078403. PubMed ID: 4923787. [Oral immunization against coli enteritis with streptomycin-dependent E. coli. V. Different efficiency of live Sm-d and **heat killed E. coli** O111 B4 **vaccine** in settling of the homologous Sm-r strain in mice with antibiotic sterilized intestine]. Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Coli-Keimen. V. Unterschiedliche Wirksamkeit von Impfstoffen aus lebenden

Streptomycin-dependenten und hitzeabgetoteten EC-O111 B4-Bakterien auf die Hemmung der Ansiedlung des homologen Streptomycin-resistenten Stammes bei darmsterilen Mausen. Lindek; Koch H. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale, **(1970)** Vol. 215, No. 3, pp. 286-95. Journal code: 0337744. ISSN: 0372-8110. L-ISSN: 0372-8110. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

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=> s vaccine carrier
L42       1455 VACCINE CARRIER

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L43          0 L42 AND KILLED E COLI

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L44         53 L42 AND E COLI

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L45          0 L44 AND DEAD

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L46          0 L44 AND ALLERGEN

=> s allergen
L47     195965 ALLERGEN

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L48       1305 L47 AND IGE EPITOPE

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L49         48 L48 AND MUTATED

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L50         10 L49 AND E COLI

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L51           3 DUP REMOVE L50 (7 DUPLICATES REMOVED)

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L51  ANSWER 1 OF 3     MEDLINE on STN                    DUPLICATE 1
2010365893.    PubMed ID: 20414052.   Expression of the recombinant major
     allergen of Salsola kali pollen (Sal k 1) and comparison with its
     low-immunoglobulin E-binding mutant. Assarehzadegan Mohammad Ali; Sankian
     Mojtaba; Jabbari Farahzad; Tehrani Mohsen; Varasteh AbdolReza. (Immunology
     Department, Faculty of Medicine, Ahvaz Jundishapur University of Medical
     Sciences, Mashhad, Iran. ) Allergology international : official journal of
     the Japanese Society of Allergology, (2010 Jun) Vol. 59, No. 2, pp.
     213-22.  Electronic Publication: 2010-04-24. Journal code: 9616296.
     E-ISSN: 1440-1592. L-ISSN: 1323-8930. Pub. country: Japan. Language:
     English.
AB   BACKGROUND: The inhalation of Salsola kali pollen is an important cause of
     pollinosis during summer and early fall throughout desert and semi-desert
     areas.  Sal k 1 has been previously reported as a major allergen
     of S. kali pollen.  In this study, we produced the recombinant Sal k 1 and
     also its low IgE-binding mutant form.  We further compared the IgE binding
     ability of these two recombinant molecules.  METHODS: The recombinant Sal
     k 1 and its low IgE-binding variant, obtained by three amino acid
     exchanges (R(142)-->S, P(143)-->A, D(144)-->V), were cloned and expressed
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in **E. coli**, as proteins fused with thioredoxin and His-tags, and then purified by Ni2+ affinity chromatography. The IgE-binding capacity of the wild-type and **mutated** rSal k 1 was compared using immunoblotting, ELISA and inhibition assays by ten sera from S. kali allergic patients. Moreover, in vivo IgE-reactivity was investigated by the skin prick test. RESULTS: Both the recombinant and the **mutated** form of Sal k 1 were expressed in **E. coli** at a relatively high amount and soluble form. All sera recognized rSal k 1 via immunoassay analysis. In addition, inhibition assays demonstrated that the purified rSal k 1 was similar to its counterpart in the crude extract. The **mutated** rSal k 1 exhibited a reduced IgE-binding capacity against wild-type rSal k 1. CONCLUSIONS: This study demonstrates that purified rSal k 1 is comprised of **IgE-epitopes** similar to that of its natural counterpart and that the **mutated** variant showed a reduced IgE-binding capacity based on in vitro assays and in vivo provocation testing.

L51 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 2

2007267167. PubMed ID: 17475857. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. Swoboda Ines; Bugajska-Schretter Agnes; Linhart Birgit; Verdino Petra; Keller Walter; Schulmeister Ulrike; Sperr Wolfgang R; Valent Peter; Peltre Gabriel; Quirce Santiago; Douladiris Nikolaos; Papadopoulos Nikolaos G; Valenta Rudolf; Spitzauer Susanne. (Institute of Medical and Chemical Laboratory Diagnostics, Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Austria.. ines.swoboda@meduniwien.ac.at) . Journal of immunology (Baltimore, Md. : 1950), (2007 May 15) Vol. 178, No. 10, pp. 6290-6. Journal code: 2985117R. ISSN: 0022-1767. L-ISSN: 0022-1767. Pub. country: United States. Language: English.

AB IgE-mediated allergy to fish is a frequent cause of severe anaphylactic reactions. Parvalbumin, a small calcium-binding protein, is the major fish **allergen**. We have recently isolated a cDNA coding for carp parvalbumin, Cyp c 1, and expressed in Escherichia coli a recombinant Cyp c 1 molecule, which contained most **IgE epitopes** of saltwater and freshwater fish. In this study, we introduced mutations into the calcium-binding domains of carp parvalbumin by site-directed mutagenesis and produced in **E. coli** three parvalbumin mutants containing amino acid exchanges either in one (single mutants; Mut-CD and Mut-EF) or in both of the calcium-binding sites (double mutant; Mut-CD/EF). Circular dichroism analyses of the purified derivatives and the wild-type **allergen** showed that Mut-CD/EF exhibited the greatest reduction of overall protein fold. Dot blot assays and immunoblot inhibition experiments performed with sera from 21 fish-allergic patients showed that Mut-CD/EF had a 95% reduced IgE reactivity and represented the derivative with the least allergenic activity. The latter was confirmed by in vitro basophil histamine release assays and in vivo skin prick testing. The potential applicability for immunotherapy of Mut-CD/EF was demonstrated by the fact that mouse IgG Abs could be raised by immunization with the **mutated** molecule, which cross-reacted with parvalbumins from various fish species and inhibited the binding of fish-allergic patients' IgE to the wild-type **allergen**. Using the hypoallergenic carp parvalbumin mutant Mut-CD/EF, it may be possible to treat fish allergy by immunotherapy.

L51 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2010 ACS on STN

2005:259357 Document No. 142:334946 Recombinant **allergens** with **mutated IgE epitopes** for treating anaphylaxis induced by food, venom, drug and latex **allergens**. Caplan, Michael J.; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 20050063994 A1 20050324, 117 pp.,

Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.

AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein **allergen**. The recombinant version can be wild-type or may include mutations within **IgE epitopes** of the protein **allergen**. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as **E. coli** . Any **allergen** may be used in the inventive methods. Particularly preferred **allergens** are anaphylactic **allergens** including protein **allergens** found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

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NEWS	3	OCT 06	Increase your retrieval consistency with new formats or for Taiwanese application numbers in CA/CAplus.

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 NEWS  4  OCT 21  CA/CAplus kind code changes for Chinese patents
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 NEWS  5  OCT 22  New version of STN Viewer preserves custom
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 NEWS  6  OCT 28  INPADOCDB/INPAFAMDB: Enhancements to the US national
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 NEWS  7  NOV 03  New format for Korean patent application numbers in
                  CA/CAplus increases consistency, saves time.
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                  December 31, 2010 by Request of Prous Science
 NEWS 10  NOV 22  Higher System Limits Increase the Power of STN
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 NEWS 12  DEC 14  New PNK Field Allows More Precise Crossover among STN
                  Patent Databases
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 NEWS 14  DEC 21  CAS Learning Solutions -- a new online training experience
 NEWS 15  DEC 22  Value-Added Indexing Improves Access to World Traditional
                  Medicine Patents in CAplus
 NEWS 16  JAN 24  The new and enhanced DPCI file on STN has been released
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=> file medline hcaplus biosis embase scisearch
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.23       0.23

FILE 'MEDLINE' ENTERED AT 13:34:18 ON 23 FEB 2011

FILE 'HCAPLUS' ENTERED AT 13:34:18 ON 23 FEB 2011
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FILE 'BIOSIS' ENTERED AT 13:34:18 ON 23 FEB 2011

FILE 'EMBASE' ENTERED AT 13:34:18 ON 23 FEB 2011

FILE 'SCISEARCH' ENTERED AT 13:34:18 ON 23 FEB 2011


```
=> s mucosal delivery
L1        1554 MUCOSAL DELIVERY

=> s l1 and E coli
L2          29 L1 AND E COLI

=> s l2 and recombinant allergen
L3           0 L2 AND RECOMBINANT ALLERGEN

=> s l2 and dead
L4           0 L2 AND DEAD

=> s l2 and killed
L5           0 L2 AND KILLED

=> s l2 and allergen
L6           0 L2 AND ALLERGEN

=> s composition
L7     4235130 COMPOSITION

=> s l7 and rectal
L8        4961 L7 AND RECTAL

=> s l8 and E coli
L9          26 L8 AND E COLI

=> s l9 and encapsulated allergen
L10          0 L9 AND ENCAPSULATED ALLERGEN

=> s l9 and allergen
L11          1 L9 AND ALLERGEN

=> s l11 and dead
L12          0 L11 AND DEAD

=> d l11 cbib abs

L11  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
2005:259357   Document No. 142:334946  Recombinant allergens with
     mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug
     and latex allergens.  Caplan, Michael J.; Bottomly, Kim H.;
     Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA).  U.S. Pat.
     Appl. Publ. US 20050063994 A1 20050324, 117 pp., Cont.-in-part of U.S.
     Ser. No. 100,303.  (English).  CODEN: USXXCO.  APPLICATION: US 2004-899551
     20040726.  PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US
     2002-100303 20020318.
AB   The present invention provides methods and compns. for treating
     or preventing allergic reactions, particularly anaphylactic reactions.
```

Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein **allergen**. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein **allergen**. Preferably the **compns.** are administered rectally. Particularly preferred microorganisms are bacteria such as **E. coli** . Any **allergen** may be used in the inventive methods. Particularly preferred **allergens** are anaphylactic **allergens** including protein **allergens** found in foods, venoms, drugs and latex. The inventive **compns.** and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

```
=> s l7 and vaginal
L13       4260 L7 AND VAGINAL

=> s l13 and E coli
L14         25 L13 AND E COLI

=> s l14 and allergen
L15          0 L14 AND ALLERGEN

=> s l14 and encapsulated
L16          0 L14 AND ENCAPSULATED

=> s l14 and dead
L17          0 L14 AND DEAD

=> s l17 and nasal
L18          0 L17 AND NASAL

=> s l7 and nasal
L19       6906 L7 AND NASAL

=> s l19 and E coli
L20         31 L19 AND E COLI

=> s l20 and allergen
L21          1 L20 AND ALLERGEN

=> s l21 and dead
L22          0 L21 AND DEAD

=> s l21 and killed
L23          0 L21 AND KILLED

=> d l21 cbib abs

L21  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
2003:610197   Document No. 139:148468  Methods and composition for
     delivering nucleic acids and/or proteins to the respiratory system.  Chen,
     Wei; Fu, Xiaoli; Nouraini, Sherry; Zhang, Zhiqing (Symbigene, Inc., USA).
     PCT Int. Appl. WO 2003063786 A2 20030807, 78 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR,
     CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
     MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG,
     SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW;
     RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB,
     GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 2003-US2469 20030127.  PRIORITY: US
```

2002-353885P 20020131; US 2002-353923P 20020131; US 2002-401465P 20020805; US 2002-280769 20021025.

AB Methods and compostions related to the fields of bacteriol., immunol. and gene therapy are provided. In general modified microflora for the delivery of vaccines, **allergens** and therapeutics to the mucosal surfaces of the respiratory tract are provided. In particular, the **compns**. and methods are directed at inducing an M-cell mediated immune response to pathogenic diseases. Specifically, methods of vaccine preparation, delivery and mucosal immunization using a Lactic Acid Bacteria (LAB), yeast and LAB that have been modified through fusion with **E**. **coli** to either present on its cell surface, or secrete, antigenic epitopes derived from pathogenic microorganisms and/or to secrete a therapeutic protein sequence are disclosed.

```
=> s l7 and oral
L24      63559 L7 AND ORAL

=> s l24 and E coli
L25        200 L24 AND E COLI

=> s l25 and allergen
L26          1 L25 AND ALLERGEN

=> s l26 and dead
L27          0 L26 AND DEAD

=> s l25 and encapsulated
L28          2 L25 AND ENCAPSULATED

=> dup remove l28
PROCESSING COMPLETED FOR L28
L29           2 DUP REMOVE L28 (0 DUPLICATES REMOVED)

=> d l29 1-2 cbib abs

L29  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2011 ACS on STN
```

2001:12293 Document No. 134:91086 Vaccine **compositions** using antigens **encapsulated** within alginate microspheres for **oral** administration and preparation process thereof. Jeong, Seo Young; Kwon, Ick Chan; Park, Joo Ae (Korea Institute of Science and Technology, S. Korea). PCT Int. Appl. WO 2001000233 A1 20010104, 64 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-KR466 19990819. PRIORITY: KR 1999-24336 19990625.

AB The present invention relates to vaccine **compns**. for **oral** administration consisting essentially of a protein antigen, in an amount effective to induce an immune response to said antigen, **encapsulated** in alginate microspheres, and to preparation thereof. More particularly, the present invention relates to vaccine **compns**. for **oral** administration consisting of a protein antigen **encapsulated** in biodegradable alginate microspheres by the diffusion-controlled interfacial gelation technique which produces microspheres having less than 5 <mm of diameter, and preparation process thereof.

L29 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2011 ACS on STN
2009:1250285 Treatment of bowel-dependent neurological disorders. Brown, Richard; Borody, Thomas Julius (Blackmores Limited, Australia). PCT Int. Appl. WO 9611014 A1 19960418 DESIGNATED STATES: W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-AU664 19951006. PRIORITY: AU 1994-8648 19941007.

AB A method is used a pharmaceutical **composition** for the treatment and/or prophylaxis of sleep disorders (narcolepsy, hypersomnia, insomnia or sleep apnoea), neuropsychiatric disorders (depression, psychosis, neurosis, catatonia, hyperactivity syndrome, manic depressive illness or anorexia nervosa), sudden infant death syndrome (SIDS), Chronic Fatigue Syndrome or children's Cl. botulinum poisoning. Said method comprises administering to subjects an effective amount of whole live or dead enteric microorganisms (selected from Bacteroides, Bifidobacterium, Eubacteria, Fusobacteria, Propionibacteria, Lactobacilli, anaerobic cocci, Ruminococcus, Escherichia, Gemmiger, Clostridium or Desulfomonas genera, a mixture of Bacteroides and **E. coli**.and so on) or cell wall containing fragments thereof (by colonoscopic infusion, enema, infusion into the small bowel via an endoscope, intubation or ingestion), further comprising removing the existing enteric microflora by administering an antibiotic and/or by bowel lavage. Said microorganisms are sonicated and **encapsulated**, in a liquid culture or are freeze dried. Said pharmaceutical **composition** further comprises one or more pharmaceutically acceptable carriers, excipients, adjuvants (one or more of gastric suppressants, H2-receptor antagonists or proton pump inhibitors) and other pharmaceutically active agents. It can be made into an **oral** dosage form and administered. Further, the method of manufacture of the said pharmaceutical **composition** includes the steps of obtaining a sample of enteric microorganisms; disrupting said microorganisms by sonication, dehydration, centrifugation, pulverisation, heating, osmotic shock, homogenisation, milling with glass beads or treatment with enzymes lysozyme; extracting from disrupted microorganisms cell wall by centrifugation, filtration or dialysis. In addition, said **composition** also has effects on treating food intolerance, irritable Bowel Syndrome symptoms, diarrhea, chronic constipation and failure to thrive.

```
=> s l7 and buccal
L30       2920 L7 AND BUCCAL

=> s l30 and E coli
L31          5 L30 AND E COLI

=> s l31 and allergen
L32          0 L31 AND ALLERGEN

=> s l31 and ovalbumin
L33          0 L31 AND OVALBUMIN

=> s l31 and dead
L34          0 L31 AND DEAD

=> s vaccine
L35     814139 VACCINE

=> s l35 and E coli
L36      11437 L35 AND E COLI
```

```
=> s l36 and recombinant allergen
L37         14 L36 AND RECOMBINANT ALLERGEN

=> s l37 and periplasm
L38          0 L37 AND PERIPLASM

=> s l37 and non-secreted
L39          0 L37 AND NON-SECRETED

=> s l37 and dead
L40          0 L37 AND DEAD

=> s l37 and heat killed
L41          0 L37 AND HEAT KILLED

=> s vaccine delivery vehicle
L42        409 VACCINE DELIVERY VEHICLE

=> s l42 and E coli
L43         19 L42 AND E COLI

=> s l43 and allergen
L44          0 L43 AND ALLERGEN

=> s l43 and dead
L45          0 L43 AND DEAD

=> s antigen presentation
L46      73061 ANTIGEN PRESENTATION

=> s l46 and dead E coli
L47          0 L46 AND DEAD E COLI

=> s l46 and E coli
L48        382 L46 AND E COLI

=> s l48 and recombinant allergen
L49          3 L48 AND RECOMBINANT ALLERGEN

=> s l49 and killed
L50          0 L49 AND KILLED

=> s l49 and dead
L51          0 L49 AND DEAD

=> dup remove l49
PROCESSING COMPLETED FOR L49
L52           2 DUP REMOVE L49 (1 DUPLICATE REMOVED)

=> d l52 1-2 cbib abs

L52  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2011 ACS on STN DUPLICATE 1
1999:581660   Document No. 132:11455  pET-prof, a plasmid for high-level
     expression of recombinant peptides fused to a birch profilin-derived
     hexadecapeptide tag: A system for the detection and presentation of
     recombinant antigens.  Pandjaitan, B.; Eibensteiner, P. B.; Vrtala, S.;
     Hayek, B.; Grote, M.; Reichelt, R.; Rumpold, H.; Valenta, R.; Spitzauer,
     S. (Vienna General Hospital, Institute of Medical and Chemical Laboratory
     Diagnostics, University of Vienna, Vienna, A-1090, Austria).  Gene,
     237(2), 333-342 (English) 1999.  CODEN: GENED6.  ISSN: 0378-1119.
     Publisher: Elsevier Science B.V..
```

AB The authors have previously identified a birch pollen profilin hexadecapeptide (Bp36/51), which was recognized by a monoclonal antibody (moAb 4A6) with high affinity. Here, they report the construction of a T7 RNA polymerase-driven high-level plasmid expression system, pET-prof, capable of producing proteins and peptides containing the Bp36/51 birch profilin-derived peptide fused to their N-terminus. As examples, the cDNAs coding for 2 major timothy grass (Phleum pratense) pollen allergens, Phl p 2 and Phl p 6, as well as for an alder (Alnus glutinosa) pollen allergen, Aln g 4, were overexpressed in Escherichia coli as BP36/51-tagged proteins. All 3 **recombinant allergens** were readily detected in nitrocellulose-blotted **E. coli** exts. by the Bp36/51-specific moAb 4A6. The authors demonstrate comparable IgE recognition of Bp36/51-tagged and untagged **recombinant allergens** by immunoblotting. A sandwich ELISA was developed using plate-bound moAb 4A6 to immobilize and present Bp36/51-tagged **recombinant allergens** to IgE antibodies of allergic patients. Using immunoelectron-microscopy, the authors demonstrate that even under harsh fixation conditions, tagged allergens can be localized simultaneously in situ by moAb 4A6 and allergen-specific antisera. The authors suggest the use of the pET-prof system for the high-level expression of Bp36/51-tagged polypeptides that can be rapidly detected in total protein exts., immunolocalized in situ, immobilized and presented to other antigen-specific antibodies (e.g. IgE), even when they occur in minute concns.

L52 ANSWER 2 OF 2 EMBASE

1996093596 EMBASE Immunologic characterization of purified recombinant timothy grass pollen (Phleum pratense) allergens (Phl p 1, Phl p 2, Phl p 5).

Vrtala, S.; Susani, M.; Sperr, W.R.; Valent, P.; Laffer, S.; Dolecek, C.; Kraft, D.; Valenta, R., Dr. (correspondence). Pathology, AKH, Inst. of General and Experimental, University of Vienna, Wahringergurtel 18-20, A-1090 Vienna, Austria.

Journal of Allergy and Clinical Immunology Vol. 97, No. 3, pp. 781-787 1996.
ISSN: 0091-6749. CODEN: JACIBY.
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 960408. Last Updated on STN: 960408

AB Background: Grass pollen allergens belong to the potent elicitors of type I allergy. Approximately 40% of allergic individuals display IgE reactivity with grass pollen allergens. In previous studies we have reported the complementary DNA cloning and expression in Escherichia coli of three of the most relevant timothy grass pollen allergens: Phl p 1, Phl p 2, and Phl p 5. Objective: To achieve high level expression of immunologically active timothy grass pollen allergens in **E. coli**, the cDNAs were inserted into expression plasmids. Methods: The three recombinant grass pollen allergens were expressed at high levels in **E. coli** as recombinant non fusion proteins, purified by conventional protein chemical methods and tested for their IgE- binding capacity by immunoblot and ELISA, as well as in histamine release assays. Results: Milligram amounts of pure **recombinant allergens** were obtained from cultured **E. coli**. IgE binding to purified recombinant Phl p 1, Phl p 2, and Phl p 5 could be demonstrated by immunoblot and ELISA. With ELISAs the percentage of grass pollen-specific IgE directed against the individual **recombinant allergens** could be estimated. In addition, the purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from patients' blood basophils. Conclusion: Purified recombinant timothy grass pollen allergens represent useful tools for diagnosis and therapy of grass pollen allergy.

```
=> s recombinant allergen
L53       3646 RECOMBINANT ALLERGEN

=> s l53 and ragweed
L54         86 L53 AND RAGWEED

=> s l54 and E coli
L55          1 L54 AND E COLI

=> s l55 and dead
L56          0 L55 AND DEAD

=> s l55 and nonsecreted
L57          0 L55 AND NONSECRETED

=> s l55 and periplasm
L58          0 L55 AND PERIPLASM

=> d l55 cbib abs

L55  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
1992:549196   Document No. 117:149196  Original Reference No. 117:25849a,25852a
     Expression and analysis of recombinant Amb a V and Amb t V allergens.
     Comparison with native proteins by immunological assays and NMR
     spectroscopy.  Rafnar, Thorunn; Ghosh, Balaram; Metzler, William J.;
     Huang, Shau Ku; Perry, Michael P.; Mueller, Luciano; Marsh, David G.
     (Johns Hopkins Asthma Allergy Cent., Baltimore, MD, 21224-6801, USA).
     Journal of Biological Chemistry, 267(29), 21119-23 (English) 1992.  CODEN:
     JBCHA3.  ISSN: 0021-9258.
AB   The Amb V allergens are small, highly disulfide-bonded ragweed
     pollen allergens that serve as useful models for understanding the mol.
     basis of the human immune response.  Recombinant Amb a V and Amb t V (from
     short and giant ragweed pollens, resp.) were produced in
     Escherichia coli and their structural and functional characteristics
     compared to those of the native proteins.  Recombinant Amb t V was
     indistinguishable from native Amb t V as determined by NMR spectroscopy and
     antibody-binding studies.  Whereas inhibition anal. showed that
     recombinant Amb a V possessed only .apprx.50% of the antibody-binding
     activity of native Amb a V, the 2 proteins were similarly effective in
     stimulating Amb a V-specific T-cells.  Thus, even highly homologous
     proteins exhibit different abilities to fold into their native
     3-dimensional conformations and the potential and limits are established
     of expressing the recombinant Amb V allergens intracellularly in E
     . coli.


=> s l53 and mugwort
L59         61 L53 AND MUGWORT

=> s l59 and E coli
L60          0 L59 AND E COLI

=> s l53 and sunflower
L61         17 L53 AND SUNFLOWER

=> s l61 and E coli
L62          6 L61 AND E COLI

=> s l62 and periplasm
L63          0 L62 AND PERIPLASM
```

```
=> s l62 and non-secreted
L64          0 L62 AND NON-SECRETED

=> s l62 and dead
L65          0 L62 AND DEAD

=> dup remove l62
PROCESSING COMPLETED FOR L62
L66           2 DUP REMOVE L62 (4 DUPLICATES REMOVED)

=> d l66 1-2 cbib abs

L66  ANSWER 1 OF 2  SCISEARCH  COPYRIGHT (c) 2011 The Thomson Corporation  on
     STN
2003:546619   The Genuine Article (R) Number: 693QZ. Peach profilin: cloning,
     heterologous expression and cross-reactivity with Bet v 2.
     Salcedo G (Reprint). ETS Ingn Agronomos, Dept Biotecnol, Unidad Bioquim,
     Ciudad Univ, Madrid 28040, Spain (Reprint). Rodriguez-Perez R;
     Fernandez-Rivas M; Gonzalez-Mancebo E; Sanchez-Monge R; Diaz-Perales A.
     ETS Ingn Agronomos, Dept Biotecnol, Unidad Bioquim, Madrid 28040, Spain;
     Fdn Hosp Alcorcon, Unidad Alergia, Madrid, Spain.
     ALLERGY (JUL 2003) Vol. 58, No. 7, pp. 635-640. ISSN: 0105-4538.
     Publisher: BLACKWELL MUNKSGAARD, 35 NORRE SOGADE, PO BOX 2148, DK-1016
     COPENHAGEN, DENMARK. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        Background: Peach is among the main foods causing allergic reactions
     in the Mediterranean adult population.  Only a single peach allergen,
     named Pru p 3, has been characterized.  However, a potential role of
     profilin has also been suggested in grass pollen-associated allergy to
     peach.
          Methods: Complementary DNA clones for two different peach profilin
     isoforms were obtained by reverse transcriptase polymerase chain reaction
     using non-degenerated primers.  Expression of recombinant peach profilin
     was performed in Escherichia coli , and confirmed using rabbit polyclonal
     antibodies to sunflower pollen profilin.  Twenty-nine individual
     sera from patients with peach allergy proved by double-blind,
     placebo-controlled food challenges (DBPCFC), either with (n = 15) or
     without (n = 14) specific IgE to Bet v 2, were used in immunodetection
     assays to test recombinant peach profilin reactivity.
          Results: Each peach profilin cDNA included an open reading frame
     coding for a 131 amino acid protein.  The peach profilin isoforms,
     designated Pru p 4.01 and Pru p 4.02, showed 80% of amino acid sequence
     identity, and were very similar (>70% identity) to allergenic profilins
     from plant foods and pollens.  Recombinant Pru p 4.01 was expressed in
     E. coli as a nonfusion protein, displaying the expected
     molecular size and reacting with anti-profilin antibodies. rPru p 4.01 was
     recognized by all sera (15 of 15) with specific IgE to Bet v 2, whereas no
     sera (zero of 14) without IgE to this birch allergen reacted with rPru p
     4.01.
          Conclusions: Peach profilin Pru p 4 is very closed to other
     allergenic profilins from plant foods and pollens.  A complete correlation
     between reactivity to rPru p 4 and rBet v 2 has been found in sera from
     peach allergic patients.

L66  ANSWER 2 OF 2     MEDLINE on STN                    DUPLICATE 1
1998184365   PubMed ID: 9525453.   Characterization of recombinant Mercurialis
     annua major allergen Mer a 1 (profilin). Vallverdu A; Asturias J A; Arilla
     M C; Gomez-Bayon N; Martinez A; Martinez J; Palacios R.
     (IFIDESA-ARISTEGUI, Research and Development Department, Bilbao, Spain. )
     The Journal of allergy and clinical immunology, (1998 Mar) Vol. 101, No.
     3, pp. 363-70.  Journal code: 1275002. ISSN: 0091-6749. L-ISSN: 0091-6749.
     Pub. country: United States. Language: English.
```

AB BACKGROUND: Two major allergens (Mer a 1A and Mer a 1B), tentatively identified as profilin, have been described in the euphorbiacea, Mercurialis annua.

OBJECTIVES: We sought to clone and characterize these major allergens from M. annua pollen and to obtain the immunologically active and soluble **recombinant allergen**, which could then be used for diagnostic procedures and therapy.

METHODS: Isolation of cDNA clones was performed by polymerase chain reaction amplification with degenerate primers. Expression in Escherichia coli BL21 (DE3) was carried out with a vector based in the T7 expression system, and the **recombinant allergen** was isolated by affinity chromatography on poly-(L-proline)-Sepharose. Electrophoretic (sodium dodecylsulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and 2-dimensional polyacrylamide gel electrophoresis) and immunochemical methods (Western blot and ELISA) were used for the characterization of the **recombinant allergen**.

RESULTS: Two cDNA inserts coding for M. annua pollen profilin (Mer a 1) were cloned and sequenced. Full-length Mer a 1 cDNA was expressed in **E. coli** as nonfusion protein. The final yield of recombinant Mer a 1 from the culture media after a single purification step on poly-(L-proline)-Sepharose was as much as 5 mg per liter. The reactivity of recombinant Mer a 1 with IgE antibodies present in sera from patients allergic to M. annua, Olea europaea, and Ricinus communis pollens was comparable to that of the natural counterparts, but latex profilin had no cross-reactivity with M. annua profilin. Recombinant Mer a 1 was shown to share B-epitopes with **sunflower** profilin.

CONCLUSION: This approach is suitable for the production of defined and purified **recombinant allergens**, which could allow more detailed immunologic characterization of these proteins and the development of much more accurate diagnostic measures and specific anti-allergic treatments.

```
=> s E coli
L67     522403 E COLI

=> s l67 and recombinant protein
L68      26748 L67 AND RECOMBINANT PROTEIN

=> s l68 and non-secreted
L69          0 L68 AND NON-SECRETED

=> s l68 and periplasm
L70        673 L68 AND PERIPLASM

=> s l70 and allergen
L71          5 L70 AND ALLERGEN

=> dup remove l71
PROCESSING COMPLETED FOR L71
L72           2 DUP REMOVE L71 (3 DUPLICATES REMOVED)

=> d l72 1-2 cbib abs

L72  ANSWER 1 OF 2     MEDLINE on STN                    DUPLICATE 1
2006113297   PubMed ID: 16499648.   Natural and recombinant molecules of the
     cherry allergen Pru av 2 show diverse structural and B cell
     characteristics but similar T cell reactivity. Fuchs H C; Bohle B;
```

Dall'Antonia Y; Radauer C; Hoffmann-Sommergruber K; Mari A; Scheiner O; Keller W; Breiteneder H. (Center of Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Mar) Vol. 36, No. 3, pp. 359-68. Journal code: 8906443. ISSN: 0954-7894. L-ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Cherry allergy is often reported in the context of allergy to other fruits of the Rosaceae family and pollinosis to trees because of cross-reactive **allergens**. Allergic reactions to cherry are reported by 19-29% of birch pollen-allergic patients. Pru av 2, identified as a thaumatin-like protein (TLP) from sweet cherry, was recognized by the majority of cherry-allergic patients in immunoblotting.

OBJECTIVES: In order to investigate the structural characteristics and the immunoglobulin (Ig)E- and T cell reactivity of cherry-derived TLP, recombinant Pru av 2 was expressed in Escherichia coli and natural Pru av 2 was purified.

METHODS: Parallel-His and FLAG expression vectors were used for recombinant production of Pru av 2 in the cytoplasm and the **periplasm** of **E. coli**. Natural Pru av 2 was purified from fresh cherries and verified by N-terminal sequencing. Structural characterization was performed using circular dichroism (CD) measurements, and the biologic activity was measured in a glucanase assay. Using cherry-specific sera, the IgE-binding ability of recombinant and natural Pru av 2 was investigated in IgE-ELISA and the T cell reactivity was studied in proliferation assays. Results Natural Pru av 2 revealed thaumatin-like structural features and bound IgE of 50% of cherry-allergic patients. It was demonstrated to be enzymatically active. Recombinant Pru av 2 expressed in the cytoplasm of **E. coli** exhibited a slightly different folding compared with the natural protein. It was not recognized by IgE from cherry-allergic subjects, but retained the ability to stimulate T lymphocytes. Periplasmic recombinant Pru av 2 was able to bind an anti-grape TLP antibody and cherry-specific IgE.

CONCLUSIONS: We prepared two recombinant model TLPs from cherry, and compared their molecular characteristics as well as their IgE-binding activity and T cell interactions in relation to the natural counterpart. The cytoplasmic recombinant Pru av 2 can be used as a hypoallergenic variant in **allergen**-specific immunotherapy, whereas the periplasmic protein can be included in a component-resolved diagnosis.

L72 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2

1995303120 PubMed ID: 7783753. Recombinant **allergen** Lol p II: expression, purification and characterization. Tamborini E; Brandazza A; De Lalla C; Musco G; Siccardi A G; Arosio P; Sidoli A. (Department of Biology and Technology, San Raffaele Scientific Institute, Milano, Italy.) Molecular immunology, (1995 May) Vol. 32, No. 7, pp. 505-13. Journal code: 7905289. ISSN: 0161-5890. L-ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Pollen from perennial rye grass (Lolium perenne) is a major cause of type I allergies worldwide. It contains complex mixtures of proteins, among which Lol p II is a major **allergen**. Previously, we have reported the cloning and sequencing of Lol p II and its expression in fusion with the heavy chain of human ferritin as carrier polypeptide (Sidoli et al., 1993, J. biol. Chemical 268, 21819-21825). Here, we describe the expression, purification and characterization of a recombinant Lol p II overproduced as a non-fusion protein in the **periplasm** of **E. coli**. The recombinant **allergen** was expressed in high yields and was easily purified in milligram amounts. It competed with the natural Lol p II for binding to

specific IgE, and it induced allergic responses in skin prick tests, indicating to be immunologically analogous to the natural protein. Biochemical analyses indicate that recombinant Lol p II is a highly stable and soluble monomeric molecule which behaves like a small globular protein.

=> s l70 and ovalbumin
L73 0 L70 AND OVALBUMIN

=> s l70 and grass
L74 3 L70 AND GRASS

=> s l74 and periplasm
L75 3 L74 AND PERIPLASM

=> dup remove l75
PROCESSING COMPLETED FOR L75
L76 1 DUP REMOVE L75 (2 DUPLICATES REMOVED)

=> d l76 cbib abs

L76 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
1995303120 PubMed ID: 7783753. Recombinant allergen Lol p II: expression, purification and characterization. Tamborini E; Brandazza A; De Lalla C; Musco G; Siccardi A G; Arosio P; Sidoli A. (Department of Biology and Technology, San Raffaele Scientific Institute, Milano, Italy.) Molecular immunology, (1995 May) Vol. 32, No. 7, pp. 505-13. Journal code: 7905289. ISSN: 0161-5890. L-ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Pollen from perennial rye **grass** (Lolium perenne) is a major cause of type I allergies worldwide. It contains complex mixtures of proteins, among which Lol p II is a major allergen. Previously, we have reported the cloning and sequencing of Lol p II and its expression in fusion with the heavy chain of human ferritin as carrier polypeptide (Sidoli et al., 1993, J. biol. Chemical 268, 21819-21825). Here, we describe the expression, purification and characterization of a recombinant Lol p II overproduced as a non-fusion protein in the **periplasm** of **E. coli**. The recombinant allergen was expressed in high yields and was easily purified in milligram amounts. It competed with the natural Lol p II for binding to specific IgE, and it induced allergic responses in skin prick tests, indicating to be immunologically analogous to the natural protein. Biochemical analyses indicate that recombinant Lol p II is a highly stable and soluble monomeric molecule which behaves like a small globular protein.

=> s l68 and stealth delivery
L77 0 L68 AND STEALTH DELIVERY

=> s l68 and antigen presenting cell
L78 19 L68 AND ANTIGEN PRESENTING CELL

=> s l78 and nonsecreted
L79 0 L78 AND NONSECRETED

=> dup remove l78
PROCESSING COMPLETED FOR L78
L80 13 DUP REMOVE L78 (6 DUPLICATES REMOVED)

=> s l80 and pd<20000406
L81 8 L80 AND PD<20000406

=> dup remove l81
PROCESSING COMPLETED FOR L81
L82 8 DUP REMOVE L81 (0 DUPLICATES REMOVED)

=> d l82 1-8 cbib abs

L82 ANSWER 1 OF 8 MEDLINE on STN
2001059616 PubMed ID: 11067933. Modulation of innate and acquired immune responses by Escherichia coli heat-labile toxin: distinct pro- and anti-inflammatory effects of the nontoxic AB complex and the enzyme activity. Ryan E J; McNeela E; Pizza M; Rappuoli R; O'Neill L; Mills K H. (Infection and Immunity Group, Institute for Immunology, National University of Ireland, Maynooth, Ireland.) Journal of immunology (Baltimore, Md. : 1950), **(2000 Nov 15)** Vol. 165, No. 10, pp. 5750-9. Journal code: 2985117R. ISSN: 0022-1767. L-ISSN: 0022-1767. Pub. country: United States. Language: English.
AB We have examined the roles of enzyme activity and the nontoxic AB complex of heat-labile toxin (LT) from Escherichia coli on its adjuvant and immunomodulatory properties. LTK63, an LT mutant that is completely devoid of enzyme activity, enhanced Th1 responses to coinjected Ags at low adjuvant dose. In contrast, LTR72, a partially detoxified mutant, enhanced Th2 responses and when administered intranasally to mice before infection with Bordetella pertussis suppressed Th1 responses and delayed bacterial clearance from the lungs. LTR72 or wild-type LT inhibited Ag-induced IFN-gamma production by Th1 cells, and LT enhanced IL-5 production by Th2 cells in vitro. Each of the toxins enhanced B7-1 expression on macrophages, but enhancement of B7-2 expression was dependent on enzyme activity. We also observed distinct effects of the nontoxic AB complex and enzyme activity on inflammatory cytokine production. LT and LTR72 suppressed LPS and IFN-gamma induced TNF-alpha and IL-12 production, but enhanced IL-10 secretion by macrophages in vitro and suppressed IL-12 production in vivo in a murine model of LPS-induced shock. In contrast, LTK63 augmented the production of IL-12 and TNF-alpha. Furthermore, LTK63 enhanced NF-kappaB translocation, whereas low doses of LTR72 or LT failed to activate NF-kappaB, but stimulated cAMP production. Thus, **E. coli** LT appears to be capable of suppressing Th1 responses and enhancing Th2 responses through the modulatory effects of enzyme activity on NF-kappaB activation and IL-12 production. In contrast, the nontoxic AB complex can stimulate acquired immune responses by activating components of the innate immune system.

L82 ANSWER 2 OF 8 MEDLINE on STN
2000278081 PubMed ID: 10816447. Extending the CD4(+) T-cell epitope specificity of the Th1 immune response to an antigen using a Salmonella enterica serovar typhimurium delivery vehicle. Lo-Man R; Langeveld J P; Deriaud E; Jehanno M; Rojas M; Clement J M; Meloen R H; Hofnung M; Leclerc C. (Unite de Biologie des Regulations Immunitaires, Institut Pasteur, Paris, France. rloman@pasteur.fr) . Infection and immunity, **(2000 Jun)** Vol. 68, No. 6, pp. 3079-89. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.
Report No.: NLM-PMC97535. Pub. country: United States. Language: English.
AB We analyzed the CD4 T-cell immunodominance of the response to a model antigen (Ag), MalE, when delivered by an attenuated strain of Salmonella enterica serovar Typhimurium (SL3261*pMalE). Compared to purified MalE Ag administered with adjuvant, the mapping of the peptide-specific proliferative responses showed qualitative differences when we used the Salmonella vehicle. We observed the disappearance of one out of eight MalE peptides' T-cell reactivity upon SL3261*pMalE immunization, but this phenomenon was probably due to a low level of T-cell priming, since it could be overcome by further immunization. The most striking effect of SL3261*pMalE administration was the activation and stimulation of new MalE

peptide-specific T-cell responses that were silent after administration of purified Ag with adjuvant. Ag presentation assays performed with MalE-specific T-cell hybridomas showed that infection of Ag-presenting cells by this intracellular attenuated bacterium did not affect the processing and presentation of the different MalE peptides by major histocompatibility complex (MHC) class II molecules and therefore did not account for immunodominance modulation. Thus, immunodominance of the T-cell response to microorganisms is governed not only by the frequency of the available T-cell repertoire or the processing steps in Ag-presenting cells that lead to MHC presentation but also by other parameters probably related to the infectious process and to the bacterial products. Our results indicate that, upon infection by a microorganism, the specificity of the T-cell response induced against its Ags can be much more effective than with purified Ags and that it cannot completely be mimicked by purified Ags administered with adjuvant.

L82 ANSWER 3 OF 8 MEDLINE on STN

2000143772 PubMed ID: 10678973. Construction and characterization of a Salmonella enterica serovar typhimurium clone expressing a salivary adhesin of Streptococcus mutans under control of the anaerobically inducible nirB promoter. Huang Y; Hajishengallis G; Michalek S M. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA.) Infection and immunity, **(2000 Mar)** Vol. 68, No. 3, pp. 1549-56. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.

Report No.: NLM-PMC97314. Pub. country: United States. Language: English.

AB Attenuated Salmonella enterica serovar Typhimurium has been used for targeted delivery of recombinant antigens to the gut-associated lymphoid tissues. One potential problem associated with this vaccine approach is the likelihood of in vivo instability of the plasmid constructs caused by constitutive hyperexpression of the heterologous immunogen. The aim of this study was to generate and characterize an expression system encoding the saliva-binding region (SBR) of Streptococcus mutans antigen I/II adhesin, either alone or linked with the mucosal adjuvant cholera toxin A2/B subunits (CTA2/B), under the control of the inducible nirB promoter. This promoter is activated in an anaerobic environment and within macrophages, which are the primary **antigen-presenting cells** involved in phagocytosis and processing of Salmonella. The gene encoding the chimeric SBR-CTA2/B was amplified by PCR using primers containing appropriate restriction sites for subcloning into pTETnirB, which contains the nirB promoter. The resulting plasmid was introduced into serovar Typhimurium by electroporation. Production of the SBR-CTA2/B chimeric protein under anaerobic conditions was verified by enzyme-linked immunosorbent assay of whole-cell lysates on plates coated with G(M1) ganglioside and developed with antibodies to SBR. Similar procedures were followed for cloning the gene encoding SBR in serovar Typhimurium under nirB control. Anaerobic expression of SBR was confirmed by Western blotting of whole-cell lysates probed with anti-SBR antibodies. The resulting serovar Typhimurium strains were administered by either the oral or the intranasal route to mice, and colonization was assessed by microbiologic analysis of dissociated spleens, Peyer's patches (PP), and nasal tissues. High numbers of the recombinant strains persisted in PP and spleen for at least 21 days following oral challenge. A single intranasal administration of the Salmonella clones to mice also resulted in the colonization of the nasal tissues by the recombinant bacteria. Salmonellae were recovered from nasal lymphoid tissues, superficial lymph nodes, internal jugular lymph nodes, PP, and spleens of mice for at least 21 days after challenge. This study provides quantitative evidence for colonization by Salmonella strains expressing a **recombinant protein** under the control of the inducible nirB promoter in PP or nasal tissues following a single oral or nasal administration of the bacteria, respectively.

L82 ANSWER 4 OF 8 MEDLINE on STN
1996342884 PubMed ID: 8720075. alpha-galactosyl (Galalpha1-3Galbeta1-4GlcNAc-R) epitopes on human cells: synthesis of the epitope on human red cells by recombinant primate alpha1,3galactosyltransferase expressed in **E.coli**.
Galili U; Anaraki F. (Department of Microbiology and Immunology, The Medical College of Pennsylvania, Philadelphia, USA.) Glycobiology, **(1995 Dec)** Vol. 5, No. 8, pp. 775-82. Journal code: 9104124. ISSN: 0959-6658. L-ISSN: 0959-6658. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Developing methods for in vitro synthesis of the carbohydrate structure Galalpha1-3Galbeta1-4GlcNAc-R (termed the alpha-galactosyl epitope) on human tumour cells may be of potential clinical significance in cancer immunotherapy. Tumour vaccines with this epitope would be opsonized in vivo by the natural anti-Gal antibody, which is present in large amounts in humans, and which interacts specifically with alpha-galactosyl epitopes. Binding of anti-Gal to alpha-galactosyl epitopes on tumour cell membranes is likely to increase uptake of the cell membranes by **antigen-presenting cells**, such as macrophages, via the adhesion of the Fc portion of anti-Gal to Fc receptors on these cells. This, in turn, may increase processing and presentation of tumour-associated antigens by **antigen-presenting cells**, and induce an effective immune response against tumour cells with these antigens. The present study describes a method for the synthesis of alpha-galactosyl epitopes on human cells (red cells used as a model) by recombinant alpha1,3galactosyltransferase (rec. alpha1,3GT) expressed in bacteria. Escherichia coli was transformed with cDNA of the luminal portion of New World monkey rec. alpha1,3GT linked to six histidines (His)6 at the N-terminus. The enzyme produced by the bacteria was isolated from bacterial lysates on a nickel-Sepharose column and eluted with imidazole. This recombinant enzyme displayed acceptor specificity similar to that of rec. alpha1,3GT produced in COS cells. Red cells were pre-treated with sialidase for exposure of N-acetyllactosamine acceptors, then subjected to rec. alpha1,3GT activity. This enzyme synthesized at least 4 x 10(4) alpha-galactosyl epitopes/red cell. These epitopes were found to be accessible for binding of anti-Gal, as well as Bandeiraea simplicifolia IB4 lectin. It is argued that the method presented can be used for the synthesis of alpha-galactosyl epitopes on membranes of autologous tumour vaccines in humans.

L82 ANSWER 5 OF 8 MEDLINE on STN
1992347390 PubMed ID: 1379182. A method for rapid screening of **recombinant proteins** for recognition by T lymphocytes.
Hickling J K; Jones K R; Yuan B; Rothbard J B; Bulow R. (ImmuLogic Pharmaceutical Corporation, Palo Alto, CA 94304.) European journal of immunology, **(1992 Aug)** Vol. 22, No. 8, pp. 1983-7. Journal code: 1273201. ISSN: 0014-2980. L-ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A simple, cost-effective method is described that allows rapid screening of **recombinant protein** sequences for their ability to stimulate T cells. Individual microcultures of **E. coli** each expressing a gene product or peptide sequence fused to protein A are grown in 96-well plates. Following lysis of the bacteria, the fusion peptide is readily captured with immobilized immunoglobulin in tissue culture wells. No further purification is required. T lymphocytes plus appropriate **antigen-presenting cells** are added directly to the wells and assayed for proliferation. The DNA in bacteria from wells stimulating T cell proliferation is then sequenced. The technique allows rapid mapping of T cell epitopes by facilitating screening of truncation mutants without extensive purification. Described here is a further application of the technique to study monosubstituted

analogues of a known T cell epitope.

L82 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2011 ACS on STN
1991:141068 Document No. 114:141068 Original Reference No. 114:23913a,23916a
Efficient recognition by rat T cell clones of an epitope of mycobacterial hsp 65 inserted in Escherichia coli outer membrane protein PhoE. Hogervorst, Els J. M.; Agterberg, Marja; Wagenaar, Josee P. A.; Adriaanse, Henriette; Boog, Claire J. P.; Van der Zee, Ruurd; Van Embden, Jan D. A.; Van Eden, Willem; Tommassen, Jan (Fac. Vet. Med., Univ. Utrecht, Utrecht, NL-3508 TD, Neth.). European Journal of Immunology, 20(12), 2763-8 (English) **1990**. CODEN: EJIMAF. ISSN: 0014-2980.
AB PhoE is a pore-forming protein, abundantly expressed in the **E. coli** outer membrane. Previous investigations have shown the possibility of inserting antigenic determinants in cell surface-exposed regions of PhoE by recombinant DNA techniques without disturbing the biogenesis and the functioning of the protein. It is now shown for the first time that PhoE can also be used as a carrier mol. for T cell epitopes. A well-characterized T cell epitope (180-188) of the 65-kDa heat-shock protein (hsp 65) of Mycobacterium tuberculosis was expressed in PhoE and tested for recognition by specific T cell clones. Specific and efficient T cell proliferation was found after stimulation with this protein construct in vitro. Interestingly, paraformaldehyde fixation of **antigen-presenting cells** did not abrogate T cell recognition. Thus, in contrast to hsp 65 itself, recognition of epitope 180-188 in the context of PhoE appeared to be independent of antigen-processing events. At the level of polyclonal T cell responses the epitope in the context of PhoE is recognized more efficiently than 180-188 as synthetic peptide or in the context of the hsp 65 mol. itself. Thus, PhoE may serve as attractive vaccine carrier not only for B, but also for T cell epitopes. Furthermore, the possibility for expression of PhoE constructs in attenuated Salmonella typhimurium strains offers the exciting prospect of new types of live oral vaccines expressing selected combinations of B and T cell epitopes.

L82 ANSWER 7 OF 8 MEDLINE on STN
1991326983 PubMed ID: 1714095. Insertion of myoglobin T-cell epitopes into the Escherichia coli alkaline phosphatase. Freimuth P; Steinman R M. (Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021.) Research in microbiology, **(1990 Sep-Oct)** Vol. 141, No. 7-8, pp. 995-1001. Journal code: 8907468. ISSN: 0923-2508. L-ISSN: 0923-2508. Pub. country: France. Language: English.
AB We are interested in antigen processing mechanisms of **antigen-presenting cells**, and to what extent the susceptibility of protein antigens to degradation contributes to immunogenicity. Understanding the biochemistry of antigen processing may be essential for reliable prediction of T-cell epitopes and for the design of vaccines that are optimized for T-cell priming. To examine possible effects of protein structural context on antigen presentation, we used genetic engineering techniques to insert helper T-cell epitopes derived from sperm whale myoglobin into surface loops of the highly stable Escherichia coli alkaline phosphatase, with the expectation that presentation of the myoglobin guest epitopes might vary with their position in the carrier protein. Levels of **recombinant protein** expression in **E. coli** cells and residual enzyme activity depended on the location of the guest peptides in the alkaline phosphatase carrier. Mutants with insertions between residues 189-190 of the carrier were recovered with yields and activities similar to the wild type protein; however, insertion of the same peptides at a second site, between residues 165-166, led to low recoveries and diminished phosphatase activities. Subcutaneous injection of mice with one of the purified **recombinant proteins** in complete Freund's adjuvant induced T cells that responded to in vitro challenge with myoglobin. The

potential use of this system to dissect processing mechanisms is discussed.

L82 ANSWER 8 OF 8 MEDLINE on STN
1989120922 PubMed ID: 3146508. Specificity of proliferative response of human CD8 clones to mycobacterial antigens. Rees A; Scoging A; Mehlert A; Young D B; Ivanyi J. (MRC Tuberculosis and Related Infections Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London, GB.) European journal of immunology, **(1988 Dec)** Vol. 18, No. 12, pp. 1881-7. Journal code: 1273201. ISSN: 0014-2980. L-ISSN: 0014-2980. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Human CD8 T lymphocyte clones (TLC) were generated from the pleural effusion of patients with tuberculosis using a protocol that required, in addition to antigen, coculture of purified CD8+ T cells, accessory cells, interleukin 2 (IL2) and anti-CD3-Sepharose. The TLC obtained were stimulated by mycobacterial soluble extracts in an IL2-dependent and MHC class I-restricted manner. When antigen-responsive TLC were screened with extracts from the recombinant mycobacterial library they were found to respond to either the Y3125 (100-kDa) or the Y3111 (71-kDa) lambda gt11 clones. Polyacrylamide gel immunoblot analysis demonstrated that the CD8 TLC responded to fractions with the molecular mass range 27-45 kDa in the Y3125 lysogen and 60-90 kDa in the mycobacterial soluble extract. The specificity of TLC reactive with the Y3111 clone was confirmed using the 71-kDa antigen purified from the same lysogen. These TLC recognized sequences common to the 71-kDa protein derived from mycobacteria, **E. coli** or a human cell line. Studies of three TLC using **antigen-presenting cells** of known genetic haplotype indicated that stimulation with both the Y3125 and the 71-kDa antigens were restricted by determinants encoded by HLA-B8.

```
=> s l70 and mugwort
L83          0 L70 AND MUGWORT

=> s l70 and sunflower profilin
L84          0 L70 AND SUNFLOWER PROFILIN

=> d his

     (FILE 'HOME' ENTERED AT 13:33:55 ON 23 FEB 2011)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 13:34:18 ON
     23 FEB 2011
L1         1554 S MUCOSAL DELIVERY
L2           29 S L1 AND E COLI
L3            0 S L2 AND RECOMBINANT ALLERGEN
L4            0 S L2 AND DEAD
L5            0 S L2 AND KILLED
L6            0 S L2 AND ALLERGEN
L7      4235130 S COMPOSITION
L8         4961 S L7 AND RECTAL
L9           26 S L8 AND E COLI
L10           0 S L9 AND ENCAPSULATED ALLERGEN
L11           1 S L9 AND ALLERGEN
L12           0 S L11 AND DEAD
L13        4260 S L7 AND VAGINAL
L14          25 S L13 AND E COLI
L15           0 S L14 AND ALLERGEN
L16           0 S L14 AND ENCAPSULATED
L17           0 S L14 AND DEAD
L18           0 S L17 AND NASAL
L19        6906 S L7 AND NASAL
```

L20 31 S L19 AND E COLI
L21 1 S L20 AND ALLERGEN
L22 0 S L21 AND DEAD
L23 0 S L21 AND KILLED
L24 63559 S L7 AND ORAL
L25 200 S L24 AND E COLI
L26 1 S L25 AND ALLERGEN
L27 0 S L26 AND DEAD
L28 2 S L25 AND ENCAPSULATED
L29 2 DUP REMOVE L28 (0 DUPLICATES REMOVED)
L30 2920 S L7 AND BUCCAL
L31 5 S L30 AND E COLI
L32 0 S L31 AND ALLERGEN
L33 0 S L31 AND OVALBUMIN
L34 0 S L31 AND DEAD
L35 814139 S VACCINE
L36 11437 S L35 AND E COLI
L37 14 S L36 AND RECOMBINANT ALLERGEN
L38 0 S L37 AND PERIPLASM
L39 0 S L37 AND NON-SECRETED
L40 0 S L37 AND DEAD
L41 0 S L37 AND HEAT KILLED
L42 409 S VACCINE DELIVERY VEHICLE
L43 19 S L42 AND E COLI
L44 0 S L43 AND ALLERGEN
L45 0 S L43 AND DEAD
L46 73061 S ANTIGEN PRESENTATION
L47 0 S L46 AND DEAD E COLI
L48 382 S L46 AND E COLI
L49 3 S L48 AND RECOMBINANT ALLERGEN
L50 0 S L49 AND KILLED
L51 0 S L49 AND DEAD
L52 2 DUP REMOVE L49 (1 DUPLICATE REMOVED)
L53 3646 S RECOMBINANT ALLERGEN
L54 86 S L53 AND RAGWEED
L55 1 S L54 AND E COLI
L56 0 S L55 AND DEAD
L57 0 S L55 AND NONSECRETED
L58 0 S L55 AND PERIPLASM
L59 61 S L53 AND MUGWORT
L60 0 S L59 AND E COLI
L61 17 S L53 AND SUNFLOWER
L62 6 S L61 AND E COLI
L63 0 S L62 AND PERIPLASM
L64 0 S L62 AND NON-SECRETED
L65 0 S L62 AND DEAD
L66 2 DUP REMOVE L62 (4 DUPLICATES REMOVED)
L67 522403 S E COLI
L68 26748 S L67 AND RECOMBINANT PROTEIN
L69 0 S L68 AND NON-SECRETED
L70 673 S L68 AND PERIPLASM
L71 5 S L70 AND ALLERGEN
L72 2 DUP REMOVE L71 (3 DUPLICATES REMOVED)
L73 0 S L70 AND OVALBUMIN
L74 3 S L70 AND GRASS
L75 3 S L74 AND PERIPLASM
L76 1 DUP REMOVE L75 (2 DUPLICATES REMOVED)
L77 0 S L68 AND STEALTH DELIVERY
L78 19 S L68 AND ANTIGEN PRESENTING CELL
L79 0 S L78 AND NONSECRETED
L80 13 DUP REMOVE L78 (6 DUPLICATES REMOVED)
L81 8 S L80 AND PD<20000406

L82 8 DUP REMOVE L81 (0 DUPLICATES REMOVED)
L83 0 S L70 AND MUGWORT
L84 0 S L70 AND SUNFLOWER PROFILIN

=> s l70 and annual mercury profilin
L85 0 L70 AND ANNUAL MERCURY PROFILIN

=> s l70 and "Amb a 1"
L86 0 L70 AND "AMB A 1"

=> s l70 and bermuda grass
L87 0 L70 AND BERMUDA GRASS

=> s l70 and orchard grass
L88 0 L70 AND ORCHARD GRASS

=> s l70 and velvet grass
L89 0 L70 AND VELVET GRASS

=> s l70 and grass
L90 3 L70 AND GRASS

=> s l90 and dead
L91 0 L90 AND DEAD

=> dup remove l90
PROCESSING COMPLETED FOR L90
L92 1 DUP REMOVE L90 (2 DUPLICATES REMOVED)

=> d l92 cbib abs

L92 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
1995303120 PubMed ID: 7783753. Recombinant allergen Lol p II: expression, purification and characterization. Tamborini E; Brandazza A; De Lalla C; Musco G; Siccardi A G; Arosio P; Sidoli A. (Department of Biology and Technology, San Raffaele Scientific Institute, Milano, Italy.) Molecular immunology, (1995 May) Vol. 32, No. 7, pp. 505-13. Journal code: 7905289. ISSN: 0161-5890. L-ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Pollen from perennial rye **grass** (Lolium perenne) is a major cause of type I allergies worldwide. It contains complex mixtures of proteins, among which Lol p II is a major allergen. Previously, we have reported the cloning and sequencing of Lol p II and its expression in fusion with the heavy chain of human ferritin as carrier polypeptide (Sidoli et al., 1993, J. biol. Chemical 268, 21819-21825). Here, we describe the expression, purification and characterization of a recombinant Lol p II overproduced as a non-fusion protein in the **periplasm** of **E. coli**. The recombinant allergen was expressed in high yields and was easily purified in milligram amounts. It competed with the natural Lol p II for binding to specific IgE, and it induced allergic responses in skin prick tests, indicating to be immunologically analogous to the natural protein. Biochemical analyses indicate that recombinant Lol p II is a highly stable and soluble monomeric molecule which behaves like a small globular protein.

=> s l70 and canary grass
L93 0 L70 AND CANARY GRASS

=> s l70 and timothy grass
L94 0 L70 AND TIMOTHY GRASS

```
=> s l70 and kentucky blue grass
L95          0 L70 AND KENTUCKY BLUE GRASS

=> s l70 and johnson grass
L96          0 L70 AND JOHNSON GRASS

=> s l70 and alder
L97          0 L70 AND ALDER

=> s l70 and birch
L98          2 L70 AND BIRCH

=> dup remove l98
PROCESSING COMPLETED FOR L98
L99           1 DUP REMOVE L98 (1 DUPLICATE REMOVED)

=> d l99 cbib abs

L99  ANSWER 1 OF 1     MEDLINE on STN                    DUPLICATE 1
2006113297   PubMed ID: 16499648.   Natural and recombinant molecules of the
     cherry allergen Pru av 2 show diverse structural and B cell
     characteristics but similar T cell reactivity. Fuchs H C; Bohle B;
     Dall'Antonia Y; Radauer C; Hoffmann-Sommergruber K; Mari A; Scheiner O;
     Keller W; Breiteneder H. (Center of Physiology and Pathophysiology,
     Medical University of Vienna, Vienna, Austria. ) Clinical and experimental
     allergy : journal of the British Society for Allergy and Clinical
     Immunology, (2006 Mar) Vol. 36, No. 3, pp. 359-68.  Journal code: 8906443.
     ISSN: 0954-7894. L-ISSN: 0954-7894. Pub. country: England: United Kingdom.
     Language: English.
AB   BACKGROUND:  Cherry allergy is often reported in the context of allergy to
     other fruits of the Rosaceae family and pollinosis to trees because of
     cross-reactive allergens.  Allergic reactions to cherry are reported by
     19-29% of birch pollen-allergic patients.  Pru av 2, identified
     as a thaumatin-like protein (TLP) from sweet cherry, was recognized by the
     majority of cherry-allergic patients in immunoblotting.

     OBJECTIVES:  In order to investigate the structural characteristics and
     the immunoglobulin (Ig)E- and T cell reactivity of cherry-derived TLP,
     recombinant Pru av 2 was expressed in Escherichia coli and natural Pru av
     2 was purified.

     METHODS:  Parallel-His and FLAG expression vectors were used for
     recombinant production of Pru av 2 in the cytoplasm and the
     periplasm of E. coli.  Natural Pru av 2 was
     purified from fresh cherries and verified by N-terminal sequencing.
     Structural characterization was performed using circular dichroism (CD)
     measurements, and the biologic activity was measured in a glucanase assay.
     Using cherry-specific sera, the IgE-binding ability of recombinant and
     natural Pru av 2 was investigated in IgE-ELISA and the T cell reactivity
     was studied in proliferation assays.  Results Natural Pru av 2 revealed
     thaumatin-like structural features and bound IgE of 50% of cherry-allergic
     patients.  It was demonstrated to be enzymatically active.  Recombinant
     Pru av 2 expressed in the cytoplasm of E. coli
     exhibited a slightly different folding compared with the natural protein.
     It was not recognized by IgE from cherry-allergic subjects, but retained
     the ability to stimulate T lymphocytes.  Periplasmic recombinant Pru av 2
     was able to bind an anti-grape TLP antibody and cherry-specific IgE.

     CONCLUSIONS:  We prepared two recombinant model TLPs from cherry, and
     compared their molecular characteristics as well as their IgE-binding
     activity and T cell interactions in relation to the natural counterpart.
     The cytoplasmic recombinant Pru av 2 can be used as a hypoallergenic
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variant in allergen-specific immunotherapy, whereas the periplasmic protein can be included in a component-resolved diagnosis.

```
=> s l70 and hornbeam
L100         0 L70 AND HORNBEAM

=> s l70 and chestnut
L101         0 L70 AND CHESTNUT

=> s l70 and hazel
L102         0 L70 AND HAZEL

=> s l70 and oak
L103         0 L70 AND OAK

=> s l70 and japonica
L104         0 L70 AND JAPONICA

=> s l70 and cedar
L105         0 L70 AND CEDAR

=> s l70 and ash
L106         0 L70 AND ASH

=> s l70 and olive
L107         0 L70 AND OLIVE

=> s l70 and lilac
L108         0 L70 AND LILAC

=> s l70 and mite
L109         0 L70 AND MITE

=> s l70 and "Der p1"
L110         0 L70 AND "DER P1"

=> s "Der p1"
L111       522 "DER P1"

=> s l111 and E coli
L112         1 L111 AND E COLI

=> d l112 cbib abs

L112 ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
2009:35733   Document No. 152:304593  Cloning and expression of group 1
     allergen of Dermatophagoides pteronyssinus.  Liu, Liang; Zhou, Ying; Cui,
     Yubao; Niu, Lina; Peng, Jianglong (Yancheng College of Medical Occupation
     and Technology, Yancheng, Jiangsu Province, 224005, Peop. Rep. China).
     Zhongguo Meijie Shengwuxue Ji Kongzhi Zazhi, 18(6), 477-482 (Chinese)
     2007.  CODEN: ZMSJB3.  ISSN: 1003-8280.  Publisher: Zhongguo Jibing Yufang
     Kongzhi Zhongxin.
AB   Objective: To clone, express and characterize Dermatophagoides
     pteronyssinus allergens.  Methods: Based on nucleotide sequence coding for
     Der p 1 in the GenBank, primers were designed and the cDNA fragment coding
     was amplified for the group 1 allergen from adult D. pteronyssinus by
     RT-PCR.  After purification and recovery, the cDNA fragment was cloned into the
     pMD19-T vector.  The fragment was then sequenced, subcloned into the
     plasmid pET28a(+), expressed in E. coli BL21 and
     identified by Western blotting.  Results: The cDNA coding for group 1
     allergen of adult D. pteronyssinus was cloned, sequenced and expressed
```

successfully. Sequence anal. showed the gene homol. with Der p 1 reported in GenBank was 99.9%, the latter encoding for a protein with 222 amino acids. Homol. anal. revealed that Der p 1 shared only 60% identity sequence with Der f 1, but Der f 1 shared 85% identity sequence with Eur m 1. Polygenetic analyses suggested that D. farinae was evolutionarily closer to Euroglyphus maynei than to D. farinae, even though D. pteronyssinus and D. farinae belonged to the same genus Dermatophagoides. Secondary structure anal. revealed that Der p 1 from China contained an alpha helix (33.78%), an extended strand (21.62%) and a random coil (44.59%). Conclusion: The cDNA coding for group 1 allergen of D. pteronyssinus were cloned, and expressed successfully, which provided a foundation for further genetic allergens product. Sequence anal. give out that D. farinae was evolutionarily closer to E. maynei than to D. pteronyssinus, which was not consistent with morphol. taxonomy adopted by most of acarologists.

```
=> s l70 and pteronyssinus
L113         0 L70 AND PTERONYSSINUS

=> s l70 and cow lipocalin
L114         0 L70 AND COW LIPOCALIN

=> s l70 and alpha-lactalbumin
L115         1 L70 AND ALPHA-LACTALBUMIN

=> d l115 cbib abs
```

L115 ANSWER 1 OF 1 EMBASE

2009283665 EMBASE Strategies for successful recombinant expression of disulfide bond-dependent proteins in Escherichia coli.

de Marco, Ario (correspondence). Cogentech, IFOM-IEO Campus for Oncogenomic, via Adamello 16, 20139 Milano, Italy. ario.demarco@ifom-ieo-campus.it.

Microbial Cell Factories Vol. 8 14 May 2009. arn. 26
Refs: 291.
. E-ISSN: 1475-2859. CODEN: MCFICT.
BioMed Central Ltd., 34 - 42 Cleveland Street, London, W1T 4LB, United Kingdom.
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20090701. Last Updated on STN: 20090701

AB Bacteria are simple and cost effective hosts for producing **recombinant proteins**. However, their physiological features may limit their use for obtaining in native form proteins of some specific structural classes, such as for instance polypeptides that undergo extensive post-translational modifications. To some extent, also the production of proteins that depending on disulfide bridges for their stability has been considered difficult in **E. coli**. Both eukaryotic and prokaryotic organisms keep their cytoplasm reduced and, consequently, disulfide bond formation is impaired in this subcellular compartment. Disulfide bridges can stabilize protein structure and are often present in high abundance in secreted proteins. In eukaryotic cells such bonds are formed in the oxidizing environment of endoplasmic reticulum during the export process. Bacteria do not possess a similar specialized subcellular compartment, but they have both export systems and enzymatic activities aimed at the formation and at the quality control of disulfide bonds in the oxidizing **periplasm**. This article reviews the available strategies for exploiting the physiological mechanisms of bactera to produce properly folded disulfide-bonded proteins. .COPYRGT. 2009 de Marco; licensee BioMed Central Ltd.

```
=> s l70 and serum albulin
L116         0 L70 AND SERUM ALBULIN

=> s l70 and cow serum albumin
L117         0 L70 AND COW SERUM ALBUMIN

=> s l70 and albumin
L118         0 L70 AND ALBUMIN

=> s 70 and immunoglobulin
L119     29580 70 AND IMMUNOGLOBULIN

=> s l119 and periplasm
L120         9 L119 AND PERIPLASM

=> dup remove l120
PROCESSING COMPLETED FOR L120
L121          4 DUP REMOVE L120 (5 DUPLICATES REMOVED)

=> d l121 1-4 cbib abs

L121 ANSWER 1 OF 4  BIOSIS  COPYRIGHT (c) 2011 The Thomson Corporation  on STN
2010:266967  Document No.: PREV201000266967. Bacterial production and
     functional characterization of the Fab fragment of the murine IgG1/lambda
     monoclonal antibody cmHsp70.1, a reagent for tumour diagnostics.
     Friedrich, Lars; Stangl, Stefan; Hahne, Hannes; Kuester, Bernhard;
     Koehler, Peter; Multhoff, Gabriele; Skerra, Arne [Reprint Author]. Tech
     Univ Munich, Munich Ctr Integrated Prot Sci, D-85350 Freising
     Weihenstephan, Germany. skerra@wzw.tum.de. Protein Engineering Design &
     Selection, (APR 2010) Vol. 23, No. 4, pp. 161-168.
     ISSN: 1741-0126. Language: English.
AB   Hsp70, the major stress-inducible member of the 70 kDa heat
     shock protein family, is frequently exposed on the plasma membrane of
     human tumours and, even more pronounced, on metastases but not detectable
     on normal tissues.  The mouse monoclonal antibody cmHsp70.1, which
     recognizes a peptide epitope in the C-terminal substrate binding domain of
     both human and murine Hsp70, provides a promising reagent for the
     monitoring of Hsp70-positive tumours during cancer therapy.  Here, we
     describe the variable domain sequences of the antibody produced by the
     hybridoma cell line and attempts to secrete the corresponding recombinant
     Fab fragment in Escherichia coli.  Initially, the yield of soluble
     functional Fab fragment that could be purified from the periplasmic cell
     extract was extremely low, even when preparing different chimeric versions
     with constant domains of human or murine origin or with the light chain
     constant domain belonging to the kappa or lambda class.  Surprisingly,
     this yield could be raised dramatically by more than a factor 100 in the
     presence of the folding helper plasmid pTUM4, which overexpresses two
     periplasmic disulphide oxido-reductases as well as two chaperones with
     proline-cis/trans-isomerase activity.  Thus, more than 15 mg functional
     recombinant Fab fragment could be purified per litre E. coli culture from
     a bench top fermenter.  This Fab fragment showed high and specific Hsp70
     binding activity in ELISA and SPR measurements, revealing a dissociation
     constant of 35 nM.  Notably, the Fab fragment sensitively recognizes the
     membrane-associated Hsp70 on tumour cell lines both in immunofluorescence
     microscopy and flow cytometry, thus showing potential for tumour detection
     in vitro and in vivo.

L121 ANSWER 2 OF 4     MEDLINE on STN                    DUPLICATE 1
2003383575   PubMed ID: 12919318.   Isolation and characterization of an IgNAR
     variable domain specific for the human mitochondrial translocase receptor
```

Tom70. Nuttall Stewart D; Krishnan Usha V; Doughty Larissa; Pearson Kylie; Ryan Michael T; Hoogenraad Nicholas J; Hattarki Meghan; Carmichael Jennifer A; Irving Robert A; Hudson Peter J. (CSIRO Health Sciences and Nutrition, Parkville, Victoria, Australia. Stewart.Nuttall@csiro.au) . European journal of biochemistry / FEBS, (2003 Sep) Vol. 270, No. 17, pp. 3543-54. Journal code: 0107600. ISSN: 0014-2956. L-ISSN: 0014-2956. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The new antigen receptor (IgNAR) from sharks is a disulphide bonded dimer of two protein chains, each containing one variable and five constant domains, and functions as an antibody. In order to assess the antigen-binding capabilities of isolated IgNAR variable domains (VNAR), we have constructed an in vitro library incorporating synthetic CDR3 regions of 15-18 residues in length. Screening of this library against the 60 kDa cytosolic domain of the **70** kDa outer membrane translocase receptor from human mitochondria (Tom70) resulted in one dominant antigen-specific clone (VNAR 12F-11) after four rounds of in vitro selection. VNAR 12F-11 was expressed into the Escherichia coli **periplasm** and purified by anti-FLAG affinity chromatography at yields of 3 mg x L(-1). Purified protein eluted from gel filtration columns as a single monomeric protein and CD spectrum analysis indicated correct folding into the expected beta-sheet conformation. Specific binding to Tom70 was demonstrated by ELISA and BIAcore (Kd = 2.2 +/- 0.31 x 10(-9) m-1) indicating that these VNAR domains can be efficiently displayed as bacteriophage libraries, and selected against target antigens with an affinity and stability equivalent to that obtained for other single domain antibodies. As an initial step in producing 'intrabody' variants of 12F-11, the impact of modifying or removing the conserved **immunoglobulin** intradomain disulphide bond was assessed. High affinity binding was only retained in the wild-type protein, which combined with our inability to affinity mature 12F-11, suggests that this particular VNAR is critically dependent upon precise CDR loop conformations for its binding affinity.

L121 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2

2002311004 PubMed ID: 12054774. Crystal structure of the anti-His tag antibody 3D5 single-chain fragment complexed to its antigen. Kaufmann Markus; Lindner Peter; Honegger Annemarie; Blank Kerstin; Tschopp Markus; Capitani Guido; Pluckthun Andreas; Grutter Markus G. (Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.) Journal of molecular biology, (2002 Apr 19) Vol. 318, No. 1, pp. 135-47. Journal code: 2985088R. ISSN: 0022-2836. L-ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB The crystal structure of a mutant form of the single-chain fragment (scFv), derived from the monoclonal anti-His tag antibody 3D5, in complex with a hexahistidine peptide has been determined at 2.7 A resolution. The peptide binds to a deep pocket formed at the interface of the variable domains of the light and the heavy chain, mainly through hydrophobic interaction to aromatic residues and hydrogen bonds to acidic residues. The antibody recognizes the C-terminal carboxylate group of the peptide as well as the main chain of the last four residues and the last three imidazole side-chains. The crystals have a solvent content of 77% (v/v) and form **70** A-wide channels that would allow the diffusion of peptides or even small proteins. The anti-His scFv crystals could thus act as a framework for the crystallization of His-tagged target proteins. Designed mutations in framework regions of the scFv lead to high-level expression of soluble protein in the **periplasm** of Escherichia coli. The recombinant anti-His scFv is a convenient detection tool when fused to alkaline phosphatase. When immobilized on a matrix, the antibody can be used for affinity purification of recombinant proteins carrying a very short tag of just three histidine residues, suitable for crystallization. The experimental structure is now the basis for the design of antibodies with even higher stability and affinity.

L121 ANSWER 4 OF 4 EMBASE COPYRIGHT (c) 2011 Elsevier B.V. All rights reserved on STN

1997248421 EMBASE Single-chain Fv antibody fragment for the immunoaffinity purification of a recombinant hepatitis B virus surface antigen particle.

Pedroso, Idolka; Agraz, Alberto; Brito, Jose; Paez, Rolando; Garcia, Jose; Perez, Mariela; Lugo, Victoria. Div. of Biopharmaceutical Devmt., Ctr. for Genetic Eng. and Biotech., Cubanacan, Playa, Havana, Cuba. Segredo, Jorge L.; Falcon, Viviana; Rodes, Lorenzo. Division of Physical Chemistry, Ctr. for Genetic Eng. and Biotech., Cubanacan, Playa, Havana, Cuba. Ayala, Marta; Freyre, Freya M.; Gavilondo, Jorge V. (correspondence). Div. Immunotechnology Diagnostics, Ctr. for Genetic Eng. and Biotech., Cubanacan, Playa, Havana, Cuba. Larrickvis@aol.com; larrickvis@aol.com. Gavilondo, Jorge V. (correspondence). Palo Alto Inst. for Molec. Medicine, 2-463 Wyandotte, St., Mountain View, CA 94043, United States. Larrickvis@aol.com; larrickvis@aol.com. Gavilondo, Jorge V. (correspondence). Palo Alto Inst. for Molecular Med., 2463 Wyandotte, St., Mountain View, CA 94043, United States. larrickvis@aol.com.

Minerva Biotecnologica Vol. 9, No. 2, pp. 68-75 Jun 1997.
Refs: 20.
ISSN: 1120-4826. CODEN: MIBIFK.
Pub. Country: Italy. Language: English. Summary Language: English.
Entered STN: 970904. Last Updated on STN: 970904

AB A single-chain Fv (scFv) antibody fragment specific for the 'a' determinant of the Hepatitis B surface antigen (HBsAg), was expressed at high levels (10-18% of the total cell protein) as an insoluble protein aggregate in the **periplasm** of E. coli MM-294 cells grown in 5-liter fermentors. The scFv was extracted from the insoluble cell material with 8 M urea and purified by immobilized metal ion affinity chromatography (IMAC) using Sepharose-IDA-Cu+2. Elution was done with 200 mM imidazole at neutral pH, with a final purity of 87%. Urea was removed by dialysis against a phosphate buffer to restore the biological activity of the scFv. This protocol yielded approximately 10 mg of pure active scFv/1 of culture. After concentration by ultrafiltration, the scFv was coupled to BrCN-activated Sepharose CL-4B at different protein/gel ratios (0.5-4.5 mg/ml of gel), and compared with the original mouse monoclonal antibody (MAb; 5.0 mg/ml of gel) as ligand for the immunoaffinity purification of a crude preparation (20% purity) of a recombinant HBsAg (r-HBsAg; average 22 nm particles) from yeast. At 4.5 mg scFv/ml of gel the antibody fragment column exhibited similar performance to the column prepared from the intact antibody, in terms of mg of eluted antigen/ml of gel, and comparable r-HBsAg purity (**70**-80%). Also, the scFv column had a similar performance to that of a MAb gel, after repetitive cycles of antigen purification. These results indicate that scFv are a viable alternative for industrial immunoaffinitty purification of large particulate antigens.

```
=> s l70 and casein
L122         6 L70 AND CASEIN

=> dup remove l122
PROCESSING COMPLETED FOR L122
L123          2 DUP REMOVE L122 (4 DUPLICATES REMOVED)

=> d l123 1-2 cbib abs
```

L123 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1

2008093546 PubMed ID: 18189353. Preparation of unglycosylated human caseinomacropeptide by engineering DAB Escherichia coli. Liu Fangjie; Liao Liang; Chen Jinchun. (College of Life Science and Technology, Beijing

University of Chemical Technology, Beijing 100029, People's Republic of China.) Journal of agricultural and food chemistry, (2008 Feb 13) Vol. 56, No. 3, pp. 889-93. Electronic Publication: 2008-01-12. Journal code: 0374755. ISSN: 0021-8561. L-ISSN: 0021-8561. Pub. country: United States. Language: English.

AB Human caseinomacropeptide (hCMP) is 65 amino acids in length and was originally derived from the C terminus of human milk kappa-**casein**. As it is highly abundant in both essential amino acids and branched amino acids, it could be developed as a practical food and even as medicinal nutrition for patients. This study was undertaken to prepare recombinant hCMP without glycosylation using recombinant plasmid and prokaryotic expression system. The gene encoding hCMP was chemically synthesized and directly inserted into the pET28a(+) vector and then expressed in Escherichia coli BL21(DE3). The maximum amount of soluble protein was obtained by incubation with 0.5 mM isopropyl-alpha-D-thiogalactopyranoside at 30 degrees C for 4 h and accounted for 40% of the total intracellular protein. Most of the expressed fusion proteins, located in the cell **periplasm** and cytoplasm, could be adsorbed by nickel affinity chromatography and eluted with buffer containing 300 mM imidazole. The fusion proteins were cleaved by enterokinase to remove the 6-His tag. Gel filtration chromatography with Sephadex G-10 was performed for desalting and purification. A final yield of 25 mg of the mature protein with high purity up to 99% was obtained from 1 L of **E. coli** culture. The purified protein was confirmed by MALDI-TOF-MS analysis. This study overcame the problem of glycosylation in hCMP and established a novel approach for the preparation of unglycosylated hCMP.

L123 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2

1991144561 PubMed ID: 1996969. Metalloendopeptidase QG. Isolation from Escherichia coli and characterization. Polgar L; Szigetvari A; Low M; Korodi I; Balla E. (Institute of Enzymology Biological Research Center, Hungarian Academy of Sciences, Budapest.) The Biochemical journal, (1991 Feb 1) Vol. 273 (Pt 3), pp. 725-31. Journal code: 2984726R. ISSN: 0264-6021. L-ISSN: 0264-6021.
Report No.: NLM-PMC1149823. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A new proteinase, which preferentially cleaves the Gln-Gly bond, was isolated from Escherichia coli. Because of this narrow specificity, the enzyme was called metalloendopeptidase QG. The proteinase is a monomer and consists of a single polypeptide chain of Mr 67,000, which is significantly smaller than the other known metalloendopeptidases of **E. coli**. It is found in the cytoplasm, but not in the **periplasm**. The enzyme cleaves the substrate benzyloxycarbonyl-Gln-Gly-Pro 2-naphthylamide between the glutamine and glycine residues, as well as its extended homologues including a nonapeptide, but it does not hydrolyse either the oxidized A and B chains of insulin or azo-**casein**. The pH-dependence of substrate hydrolysis gives a bell-shaped curve with pK1 = 6.6 and pK2 = 8.8. The metallopeptidase is inhibited in Tris and imidazole buffers, the basic components of which are presumably liganded to the essential Zn2+ ion. 2-Aminobenzoyl-Gln-Gly-Pro 2-naphthylamide, designed as a fluorescent substrate for the metallopeptidase, proved to be a strong inhibitor. Bestatin, an inhibitor of aminopeptidases in the micromolar concentration range, inhibits the metalloendopeptidase only in the millimolar concentration range. Captopril, the widely used inhibitor of angiotensin-converting enzyme, is a fairly good inhibitor of the metalloendopeptidase. The simplest inhibitor that can be used to protect **recombinant proteins** from degradation by the metalloendopeptidase may be EDTA, which is effective at low millimolar concentration.

```
=> s l70 and albumin
L124         0 L70 AND ALBUMIN

=> s l70 and lipocalin
L125        15 L70 AND LIPOCALIN

=> s l125 and pd<20000406
L126         3 L125 AND PD<20000406

=> dup remove l126
PROCESSING COMPLETED FOR L126
L127          3 DUP REMOVE L126 (0 DUPLICATES REMOVED)

=> d l127 1-3 cbib abs

L127 ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2011 ACS on STN
1999:184566   Document No. 131:4030  Complement component C8γ is
     expressed in human fetal and adult kidney independent of C8α.
     Trojer, Patrick; Wojnar, Petra; Merschak, Petra; Redl, Bernhard (Institut
     fur Mikrobiologie (Med. Fakultat), Universitat Innsbruck, Innsbruck,
     A-6020, Austria).  FEBS Letters, 446(2,3), 243-246 (English) 1999
     .  CODEN: FEBLAL.  ISSN: 0014-5793.  Publisher: Elsevier Science B.V..
AB   Human complement component C8γ is an unusual complement factor since
     it shows no homol. to other complement proteins but is a member of the
     lipocalin superfamily.  So far, it has been found exclusively in
     plasma, covalently linked to C8α by disulfide bridging.  We have
     used dot blot and Northern blot analyses of a large number of different human
     tissues to survey systematically the expression pattern of C8γ.  Our
     expts. clearly showed that besides in liver, this gene is also expressed
     in fetal and adult kidney.  Renal expression of C8γ is not dependent
     on C8α expression, since we could not detect C8α expression in
     kidney.  Thus, its physiol. function is not restricted to a specific
     action in association with complement components.  As a prerequisite for
     further characterization of the structure and binding activities of the
     uncomplexed C8γ, we have expressed the encoding cDNA in Escherichia
     coli.  To increase the probability for proper folding of the
     characteristic intramol. disulfide bridge the recombinant
     protein was produced by secretion to the periplasm.

L127 ANSWER 2 OF 3     MEDLINE on STN
1999288215   PubMed ID: 10336862.   Expression of a lipocalin in
     prokaryote and eukaryote cells: quantification and structural
     characterization of recombinant bovine beta-lactoglobulin. Chatel J M;
     Adel-Patient K; Creminon C; Wal J M. (INRA-CEA, CEA, Laboratoire d'Etudes
     RadioImmunologique, DRM-SPI, Bat 136, CE Saclay, Gif Sur Yvette, 91191,
     France. chatel@dsvidf2@cea.fr) . Protein expression and purification,
     (1999 Jun) Vol. 16, No. 1, pp. 70-5.  Journal code: 9101496. ISSN:
     1046-5928. L-ISSN: 1046-5928. Pub. country: United States. Language:
     English.
AB   In this paper we quantify and characterize the expression of recombinant
     beta-lactoglobulin (rBLG) in prokaryote and eukaryote cells.  In
     Escherichia coli we used the pET26 vector, which permits the secretion of
     rBLG in periplasm.  We studied the expression of rBLG in COS-7
     cells and in vivo in mouse tibialis muscle.  The expression of rBLG was
     measured by two immunoassays specific, respectively, for BLG in its native
     and denatured conformation.  We observed that rBLG was essentially
     expressed in a denatured form in E. coli even in the
     periplasm, whereas rBLG in eukaryote cells was found in its native
     conformation.
     Copyright 1999 Academic Press.
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L127 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2011 ACS on STN
1993:423106 Document No. 119:23106 Original Reference No. 119:4185a,4188a
Functional expression of the uncomplexed serum retinol-binding protein in Escherichia coli. Ligand binding and reversible unfolding characteristics.
Mueller, Holger N.; Skerra, Arne (Max-Planck-Inst. Biophys., Frankfurt/Main, W-6000/71, Germany). Journal of Molecular Biology, 230(3), 725-32 (English) **1993**. CODEN: JMOBAK. ISSN: 0022-2836.

AB The serum retinol-binding protein solubilizes the lipophilic vitamin A alc. and plays an important physiol. role in the transport of this compound The monomeric single-domain protein, the three-dimensional structure of which is known, constitutes a well-characterized member of the **lipocalin** family of proteins. The functional expression of the apo-protein in **E. coli** by secretion to the **periplasm** is reported here. The **recombinant protein**, purified in a single step by metal chelate affinity chromatog., exhibits the same ligand binding characteristics as described for the natural protein. Guanidinium chloride-induced unfolding and refolding expts. suggest that the recombinant retinol-binding protein adopts a stable conformation despite being expressed and purified in the absence of the large hydrophobic ligand. The expression system described here should also be useful for the recombinant production of other **lipocalin** proteins, thus permitting the elucidation of the structure-function relationships of ligand binding by protein engineering.

```
=> s l70 and "Fel d1"
L128         0 L70 AND "FEL D1"

=> s l70 and cat antigen
L129         0 L70 AND CAT ANTIGEN

=> s l70 and MUP antigen
L130         0 L70 AND MUP ANTIGEN

=> s l70 and fungus antigen
L131         0 L70 AND FUNGUS ANTIGEN

=> s l70 and heat shocl protein
L132         0 L70 AND HEAT SHOCL PROTEIN

=> s l70 and YCP4
L133         0 L70 AND YCP4

=> s l70 and c herbarum
L134         0 L70 AND C HERBARUM

=> s l70 and fugus
L135         0 L70 AND FUGUS

=> s l70 and fungus
L136         2 L70 AND FUNGUS

=> dup remove l136
PROCESSING COMPLETED FOR L136
L137          2 DUP REMOVE L136 (0 DUPLICATES REMOVED)

=> s l137 and pd<20000406
L138         1 L137 AND PD<20000406

=> d l138 cbib abs

L138 ANSWER 1 OF 1     MEDLINE on STN
```

1989137080 PubMed ID: 2645134. Proteinase K from Tritirachium album Limber. Characterization of the chromosomal gene and expression of the cDNA in Escherichia coli. Gunkel F A; Gassen H G. (Institut fur Biochemie, Technische Hochschule Darmstadt, Federal Republic of Germany.) European journal of biochemistry / FEBS, **(1989 Jan 15)** Vol. 179, No. 1, pp. 185-94. Journal code: 0107600. ISSN: 0014-2956. L-ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB The cDNA and the chromosomal gene encoding proteinase K from Tritirachium album Limber have been cloned in Escherichia coli and the entire nucleotide sequences of the coding region, as well as 5'- and 3'-flanking regions have been determined. The deduced primary translation product consisting of 384 amino acid residues (molecular mass = 40,231 Da) contains an N-terminal region of 105 amino acids not present in the mature protein. By analogy to the evolutionary-related bacterial subtilisins and other serine proteinases it is inferred that the primary secreted product is a zymogen containing a 15-amino-acid signal sequence and a 90-amino-acid propeptide. The propeptide is presumably removed in the later steps of the secretion process or upon secretion into the medium. The nucleotide-sequence analysis of the gene and its flanking regions has revealed that the proteinase-K gene is composed of two exons and one 63-bp-long intron located in the proregion. Furthermore, a putative promoter sequence and a capping site have been identified, suggesting that the transcription-start site is located 103-bp upstream of the ATG initiation codon. To express the proproteinase-K gene in **E. coli**, proproteinase-K cDNA was cloned in a plasmid vector under control of the tac promoter. The hybrid plasmid pSPPRO, constructed for this purpose, contained the cDNA coding for proproteinase K [from Ala (-91) to the C-terminal Ala (279)] fused to the N-terminal-signal-peptide sequence of the alkaline-phosphatase gene preceded by the tac promoter. **E. coli** BMH71-18, harbouring this plasmid, exhibited slight proteolytic activity when tested on skimmed-milk plates, suggesting that some fusion proteins were correctly secreted into the **periplasm** and processed to the mature proteinase K.

```
=> s l70 and shaggy cap
L139         0 L70 AND SHAGGY CAP

=> s l70 and coprinus comatus
L140         0 L70 AND COPRINUS COMATUS

=> s l70 and apyrase
L141         0 L70 AND APYRASE

=> s l41 and mosquito
L142         0 L41 AND MOSQUITO

=> s l70 and Aed
L143         0 L70 AND AED

=> s l70 and honey bee
L144         0 L70 AND HONEY BEE

=> s l70 and phospholipase
L145         3 L70 AND PHOSPHOLIPASE

=> s l145 and bee
L146         0 L145 AND BEE

=> dup remove l145
PROCESSING COMPLETED FOR L145
```

L147 3 DUP REMOVE L145 (0 DUPLICATES REMOVED)

=> d l147 1-3 cbib abs

L147 ANSWER 1 OF 3 MEDLINE on STN
2001432164 PubMed ID: 11478968. Expression of an active recombinant lysine 49 **phospholipase** A(2) myotoxin as a fusion protein in bacteria.
Giuliani C D; Iemma M R; Bondioli A C; Souza D H; Ferreira L L; Amaral A C; Salvini T F; Selistre-de-Araujo H S. (Departamento de Ciencias Fisiologicas, Universidade Federal de Sao Carlos, 13565 Sao Carlos, SP, Brazil.) Toxicon : official journal of the International Society on Toxinology, (2001 Oct) Vol. 39, No. 10, pp. 1595-600. Journal code: 1307333. ISSN: 0041-0101. L-ISSN: 0041-0101. Pub. country: England: United Kingdom. Language: English.
AB ACL myotoxin (ACLMT) is a K49 **phospholipase** A(2)-like protein isolated from the venom of the snake Agkistrodon contortrix laticinctus (broad-banded copperhead) that induces necrosis of skeletal muscle. We have previously cloned and sequenced the cDNA coding for ACLMT from a venom gland cDNA library. In order to perform structure and function studies, we have developed an expression system for production of ACLMT as a fusion protein with maltose binding protein (MBP) from the **periplasm** of bacteria, using the pMAL-p2 expression vector. The cDNA coding for the mature toxin without the signal peptide was amplified by PCR and subcloned into the pMAL-p2 vector. The new plasmid (pMAL-MT) was used to transform BL21(DE3) **E. coli** cells.
Culture of transformed cells induced with IPTG led to the expression of a 60 kDa fusion protein which strongly reacts with anti-native ACLMT antibodies. The fusion protein was purified from the bacterial **periplasm** by affinity chromatography in an amylose column and by gel filtration. The purified fusion protein (MBP-rACLMT) was able to induce necrosis of skeletal muscle of mice very similar to that caused by the native myotoxin.

L147 ANSWER 2 OF 3 MEDLINE on STN
1998129912 PubMed ID: 9468660. Release of miniantibodies from **E. coli** cells into the supernatant at low and high cell densities.
Morbe J L; Riesenberg D. (Institute of Toxicology, ETH, Schwerzenbach, Switzerland.) Microbiological research, (1997 Dec) Vol. 152, No. 4, pp. 385-94. Journal code: 9437794. ISSN: 0944-5013. L-ISSN: 0944-5013. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB By chemical permeabilization of **E. coli** cells with detergents and membrane active peptides considerable amounts of the periplasmic anti-PC-miniantibodies can be released. Releases of active miniantibodies of 31%, respectively of 38%, were obtained by exposing low cell density suspensions to nonionic detergents like Triton X-100 and tetraethyleneglycolmonodecylether. At high cell densities releasing levels of 40% and 56% were observed for these detergents. The addition of cationic, membrane active peptides (PMBN, polylysine 115, magainin II and melittin) led to release of up to 20% of active miniantibodies at low cell densities. The excretion of miniantibodies at low cell densities was increased up to 35% by **phospholipase** A2. Interestingly, the membrane associating properties of the anti-PC-miniantibodies influenced the permeability of the outer membrane and the excretion of beta-lactamase.

L147 ANSWER 3 OF 3 MEDLINE on STN
1989313290 PubMed ID: 2546005. **Phospholipase** C and haemolytic activities of Clostridium perfringens alpha-toxin cloned in Escherichia coli: sequence and homology with a Bacillus cereus **phospholipase** C.
Leslie D; Fairweather N; Pickard D; Dougan G; Kehoe M. (Department of Microbiology, University of Newcastle upon Tyne, Medical School, Framlington Place, UK.) Molecular microbiology, (1989 Mar) Vol. 3, No. 3,

pp. 383-92. Journal code: 8712028. ISSN: 0950-382X. L-ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The Clostridium perfringens alpha-toxin (**phospholipase** C) gene (cpa) has been cloned and expressed in Escherichia coli. The biological activities of the cloned gene product have been analysed and the complete nucleotide sequence of the cpa gene has been determined. The cloned cpa gene product, which is exported to the **periplasm** in **E. coli**, possesses both **phospholipase** C and haemolytic activities. Haemolysis is not apparent when cell extracts are incubated with isotonic suspensions of sheep erythrocytes, but can be detected and quantified readily when dilutions of the same extracts are placed in wells in sheep-blood agar plates. Like other sequenced clostridial genes, the cpa gene has a high AT content (66.4%), exhibits a strong bias for using codons with A or T in the wobble position, and the 350 base pairs upstream from the gene have a significantly higher AT content (79.5%) than the coding region. The cpa gene encodes a 398 amino acid polypeptide with a deduced molecular weight of 45,481 D. This is very similar to the estimated molecular weight (Mr) of the cpa primary gene product expressed in an in vitro transcription-translation system (Mr 46,000), but larger than the cpa gene product detected in **E. coli** minicells, **E. coli** whole cells or in C. perfringens cells (Mr 43,000), suggesting post-translational processing. The 28 N-terminal residues of the deduced alpha-toxin sequence possess the consensus features of a signal peptide and may be removed during secretion. The deduced alpha-toxin sequence shares significant structural homology with the phosphatidylcholine-preferring **phospholipase** C of Bacillus cereus.

```
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L148        40 L70 AND TOXIN

=> s l48 and non-secreted
L149         0 L48 AND NON-SECRETED

=> s l148 and not secrete
MISSING TERM 'AND NOT'
The search profile that was entered contains a logical
operator followed immediately by another operator.

=> s l148 and encapsulated
L150         0 L148 AND ENCAPSULATED

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L151        22 L148 AND PD<20000406

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PROCESSING COMPLETED FOR L151
L152         12 DUP REMOVE L151 (10 DUPLICATES REMOVED)

=> s l152 and dead
L153         0 L152 AND DEAD

=> d l152 1-12 cbib abs

L152 ANSWER 1 OF 12     MEDLINE on STN
1998013106   PubMed ID: 9353057.   Exclusion of bioactive contaminations in
     Streptococcus pyogenes erythrogenic toxin A preparations by
     recombinant expression in Escherichia coli. Fagin U; Hahn U; Grotzinger J;
     Fleischer B; Gerlach D; Buck F; Wollmer A; Kirchner H; Rink L. (Institute
     of Immunology and Transfusion Medicine, University of Lubeck School of
     Medicine, Germany. ) Infection and immunity, (1997 Nov) Vol. 65,
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No. 11, pp. 4725-33. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.
Report No.: NLM-PMC175678. Pub. country: United States. Language: English.

AB The streptococcal erythrogenic exotoxin A (SPEA) belongs to the family of bacterial superantigens and has been implicated in the pathogenesis of a toxic shock-like syndrome and scarlet fever. Concerning its biological activity, mainly T-cell-stimulatory properties, conflicting data exist. In this study, we show that most of the SPEA preparations used so far contain biologically active contaminations. Natural SPEA from the culture supernatant of Streptococcus pyogenes NY-5 and recombinant SPEA purified from the culture filtrate of S. sanguis are strongly contaminated with DNases. We show that natural SPEA induces more tumor necrosis factor alpha (TNF-alpha) than recombinant SPEA, but we also show that DNases are able to induce TNF-alpha. In commercial SPEA preparations, we identified a highly active protease, which was shown not to be SPEB. To exclude these contaminations, we overexpressed SPEA cloned in the effective high-level expression vector pIN-III-ompA2 in Escherichia coli. The expressed SPEA shows the same amino acid composition as natural SPEA, whereas functional studies reported so far were carried out with **toxins** containing an incorrect amino terminus. We describe the rapid purification of lipopolysaccharide-, DNase-, and protease-free SPEA in two steps from the host's **periplasm** and its structural characterization by circular dichroism. Our results represent for the first time the production in **E. coli** of recombinant SPEA with the authentic N-terminal sequence and a proven superantigenic activity. Collectively, our results indicate that immunological studies of superantigens require highly purified substances free of biologically active contaminations.

L152 ANSWER 2 OF 12 MEDLINE on STN DUPLICATE 1

1998096687 PubMed ID: 9435018. Construction and characterization of versatile cloning vectors for efficient delivery of native foreign proteins to the **periplasm** of Escherichia coli. Jobling M G; Palmer L M; Erbe J L; Holmes R K. (Department of Microbiology, University of Colorado Health Sciences Center, Denver 80262, USA.) Plasmid, **(1997)** Vol. 38, No. 3, pp. 158-73. Journal code: 7802221. ISSN: 0147-619X. L-ISSN: 0147-619X. Pub. country: United States. Language: English.

AB Induction of the wild type cholera **toxin** operon (ctxAB) from multicopy clones in Escherichia coli inhibited growth and resulted in low yields of cholera **toxin** (CT). We found that production of wild type CT or its B subunit (CT-B) as a periplasmic protein was toxic for **E. coli**, but by replacing the native signal sequences of both CT-A and CT-B with the signal sequence from the B subunit of **E. coli** heat-labile enterotoxin LTIIb we succeeded for the first time in producing CT holotoxin in high yield in **E. coli**. Based on these findings, we designed and constructed versatile cloning vectors that use the LTIIb-B signal sequence to direct recombinant native proteins with high efficiency to the **periplasm** of **E. coli**. We confirmed the usefulness of these vectors by producing two other secreted **recombinant proteins**. First, using phoA from **E. coli**, we demonstrated that alkaline phosphatase activity was 17-fold greater when the LTIIb-B signal sequence was used than when the native leader for alkaline phosphatase was used. Second, using the pspA gene that encodes pneumococcal surface protein A from Streptococcus pneumoniae, we produced a 299-residue amino-terminal fragment of PspA in **E. coli** in large amounts as a soluble periplasmic protein and showed that it was immunoreactive in Western blots with antibodies against native PspA. The vectors described here will be useful for further studies on structure-function relationships and vaccine development with CT and PspA, and they should be valuable as general tools for delivery of other

secretion-competent **recombinant proteins** to the **periplasm** in **E. coli.**

L152 ANSWER 3 OF 12 MEDLINE on STN

1998294412 PubMed ID: 9630956. Translational level is a critical factor for the secretion of heterologous proteins in Escherichia coli. Simmons L C; Yansura D G. (Department of Molecular Biology, Genentech, Inc., South San Francisco, CA 94080, USA. simmons@gene.com) . Nature biotechnology, **(1996 May)** Vol. 14, No. 5, pp. 629-34. Journal code: 9604648. ISSN: 1087-0156. L-ISSN: 1087-0156. Pub. country: United States. Language: English.

AB A method for enhancing the secretion of heterologous proteins in Escherichia coli by optimizing, as opposed to simply maximizing, the translational level of a given protein is described. Random alteration of the translational initiation region (TIR) of the Heat-Stable Enterotoxin II (STII) signal sequence resulted in a library of vectors with varied translational strengths. Subsequent screening of this library using **E. coli** alkaline phosphatase as a reporter led to the selection of several TIR variants covering a 10-fold range of translational strength. These TIR variants, in combination with several previously generated variants, are shown to dramatically improve the secretion of a number of heterologous proteins. In fact, the heterologous proteins tested required a narrow translational range for optimal high-level secretion into the **periplasm**. Interestingly, the secretion of two native **E. coli** proteins was unaffected by TIR strength when tested over an identical range. The dependence of secretion on a narrow translational level demonstrates its critical role in the secretion of heterologous proteins in **E. coli.**

L152 ANSWER 4 OF 12 MEDLINE on STN DUPLICATE 2

1997080554 PubMed ID: 8921899. Absence of periplasmic DsbA oxidoreductase facilitates export of cysteine-containing passenger proteins to the Escherichia coli cell surface via the Iga beta autotransporter pathway. Jose J; Kramer J; Klauser T; Pohlner J; Meyer T F. (Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany.) Gene, **(1996 Oct 31)** Vol. 178, No. 1-2, pp. 107-10. Journal code: 7706761. ISSN: 0378-1119. L-ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB The Iga beta autotransporter function of IgA1 protease from Neisseria gonorrhoeae was assessed in Escherichia coli using the Vibrio cholerae **toxin** B subunit (CtxB) as a heterologous passenger. N-terminal fusions with Iga beta of native CtxB or mutant CtxB protein containing no cysteines were constructed and analysed in isogenic **E. coli** mutants carrying defects in either or both the ompT (outer membrane protease T) and dsbA (periplasmic disulfide oxidoreductase) determinants. While export of the cystein-less CtxB passenger was independent of the dsbA genotype, the native CtxB passenger was properly translocated across the outer membrane only in the dsbA mutant background. This effect was consistent in the presence and in the absence of the OmpT protease which rather determined the release of surface-bound CtxB into the medium. Therefore, in agreement with previous observations Iga beta-dependent protein secretion requires an unfolded conformation of the passenger domain and can be blocked by disulfide loop formation in the presence of DsbA. Since DsbA acts in the **periplasm**, this provides evidence for a periplasmic intermediate in the Iga beta-mediated export pathway. **E. coli** (dsbA ompT) is highly suitable as a strain for the surface display of **recombinant proteins** via Iga beta, whether or not they contain cysteine residues.

L152 ANSWER 5 OF 12 MEDLINE on STN

1996193914 PubMed ID: 8641438. Formation of disulfide bonded dimer of mutated heat-labile enterotoxin in vivo. Hedges P A; Hardy S J. (Department of Biology, University of York, UK.) FEBS letters, **(1996 Feb 26)** Vol. 381, No. 1-2, pp. 53-6. Journal code: 0155157. ISSN: 0014-5793. L-ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB One of the two cysteines in the B subunit of heat-labile enterotoxin has been changed to a serine by site-directed mutagenesis so that the internal disulfide bond cannot form. The mutant protein, like the wild-type protein synthesised in the presence of the reducing agent dithiothreitol, does not form pentamers in the **periplasm** but binds to available membranes. Binding to membranes is disrupted by chaotropic agents but not by salt. More than half the molecules of mutant protein form disulfide-bonded dimers when exported to the **periplasm** but no dimer is detected when the protein is exported to the medium by spheroplasts.

L152 ANSWER 6 OF 12 HCAPLUS COPYRIGHT 2011 ACS on STN

1996:507446 Document No. 125:299447 Original Reference No. 125:56055a,56058a Large scale recovery and purification of periplasmic **recombinant protein** from **E. coli** using expanded bed adsorption chromatography followed by new ion exchange media. Johansson, H. J.; Jaegersten, C.; Shiloach, J. (Pharmacia Biotech AB, Uppsala, S-75182, Swed.). Journal of Biotechnology, 48(1,2), 9-14 (English) **1996.** CODEN: JBITD4. ISSN: 0168-1656. Publisher: Elsevier.

AB Expanded bed chromatog. was used for the recovery and purification of modified Pseudomonas aeruginosa exotoxin A. The exotoxin accumulates in the periplasmic space of **E. coli** BL21(λDE3), was released from the cells by osmotic shock and captured by applying the open cell suspension directly to an anion exchanger (STREAMLINE DEAE) using an expanded bed (STREAMLINE) column. Processing of 4.5 kg of **E. coli** using the expanded bed process was 3 times faster and did not require clarification of the bacterial extract, in comparison with the conventional purification method. Also, the recovered protein solution was 3 times more concentrated and the yield slightly higher.

L152 ANSWER 7 OF 12 MEDLINE on STN DUPLICATE 3

1995310042 PubMed ID: 7790099. Different forms of streptolysin O produced by Streptococcus pyogenes and by Escherichia coli expressing recombinant **toxin**: cleavage by streptococcal cysteine protease. Pinkney M; Kapur V; Smith J; Weller U; Palmer M; Glanville M; Messner M; Musser J M; Bhakdi S; Kehoe M A. (Department of Microbiology, Medical School, University of Newcastle, Newcastle upon Tyne, United Kingdom.) Infection and immunity, **(1995 Jul)** Vol. 63, No. 7, pp. 2776-9. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567. Report No.: NLM-PMC173373. Pub. country: United States. Language: English.

AB To resolve apparent discrepancies in the literature, N-terminal sequences of the active high- and low-molecular-weight (high- and low-M(r)) forms of native streptolysin O (nSLO) purified from Streptococcus pyogenes culture supernatants and of the similar-size high- and low-M(r) forms of recombinant SLO (rSLO) found in the **periplasm** of Escherichia coli expressing a cloned slo gene were determined. The high-M(r) forms of nSLO and rSLO are identical, reflecting removal of a 31-residue signal peptide, but the similar-size low-M(r) forms are very different. Removal of C-terminal sequences by proteases in the **E. coli periplasm** produces an inactive low-M(r) form of rSLO. In contrast, an active low-M(r) form of nSLO is produced by proteolytic cleavage between the N-terminal residues Lys-77 and Leu-78, which was shown to correspond to an extremely sensitive cleavage site for the pyrogenic exotoxin B-derived streptococcal cysteine protease.

L152 ANSWER 8 OF 12 MEDLINE on STN

1992335266 PubMed ID: 1631109. pH-dependent insertion of proteins into membranes: B-chain mutation of diphtheria **toxin** that inhibits membrane translocation, Glu-349----Lys. O'Keefe D O; Cabiaux V; Choe S; Eisenberg D; Collier R J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.) Proceedings of the National Academy of Sciences of the United States of America, **(1992 Jul 1)** Vol. 89, No. 13, pp. 6202-6. Journal code: 7505876. ISSN: 0027-8424. L-ISSN: 0027-8424.
Report No.: NLM-PMC402150. Pub. country: United States. Language: English.

AB To investigate how diphtheria **toxin** (DT) undergoes pH-dependent membrane translocation in mammalian cells, we have isolated and characterized mutants of the **toxin** that are defective in acidic-pH-dependent killing of Escherichia coli. Cloned DT secreted to the **periplasm** of **E. coli** kills the bacteria under acidic conditions (near pH 5.0) by inserting into and permeabilizing the inner membrane (a mechanism independent of the **toxin**'s ADP-ribosylation activity). Mutant forms of DT with reduced lethality for **E. coli** were selected by plating the bacteria under acidic conditions. CRM503, one of the full-length mutants selected by this protocol, also showed diminished cytotoxicity for mammalian cells. We traced the altered cytotoxicity of CRM503 to a Glu-349----Lys mutation (E349K), one of three point mutations, within the B fragment. The E349K mutation alone inhibited cytotoxicity and membrane translocation in mammalian cells and lethality for **E. coli** but did not affect enzymic activity or receptor binding. The recently determined crystallographic model of DT shows that Glu-349 resides within a short loop connecting two long hydrophobic alpha-helices of the translocation domain. Protonation of Glu-349 and two other nearby acidic residues, Asp-352 and Glu-362, may enable these helices to undergo membrane insertion and the intervening loop to be transferred to the opposite face of the bilayer. The E349K mutation introduces a positive charge at this site, which would be expected to inhibit membrane insertion and the insertion-dependent activities of DT. These results suggest that protonation of Glu-349 and nearby acidic residues may be important in triggering the translocation step of **toxin** action.

L152 ANSWER 9 OF 12 MEDLINE on STN

1990215972 PubMed ID: 2108926. Secretion of **toxin** A from Pseudomonas aeruginosa PAO1, PAK, and PA103 by Escherichia coli. Hamood A N; Wick M J; Iglewski B H. (Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, New York 14642.) Infection and immunity, **(1990 May)** Vol. 58, No. 5, pp. 1133-40. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.
Report No.: NLM-PMC258600. Pub. country: United States. Language: English.

AB The exotoxin A gene (toxA) from Pseudomonas aeruginosa PAO1 was expressed from the lac promoter in Escherichia coli, and the localization of the **toxin** A protein was determined. Throughout the growth cycle, the ADP-ribosyltransferase activity of **toxin** A was gradually reduced in the **periplasm** of **E. coli**, with no apparent degradation of the **toxin** A protein. This suggests the presence of an **E. coli** periplasmic factor that interferes with the ADP-ribosyltransferase activity in **toxin** A. Such an inactivating factor was found in the periplasmic extract from control **E. coli** cells. The processing of **toxin** A in **E. coli** was examined by pulse-chase immunoprecipitation experiments. Mature **toxin** was detected in both the **periplasm** and cytoplasm, whereas the membranes contained both mature and precursor forms. **Toxin** A precursor appears to be processed in both the cytoplasm and the **periplasm** of **E. coli**. **Toxin** A proteins from P. aeruginosa PAO1, PA103, and PAK were compared for their secretion in **E. coli**. Despite the differences in the amino acid

sequences of their leader peptides, **toxin** A proteins from strains PAO1, PA103, and PAK were processed and secreted to the **periplasm** of **E. coli**.

L152 ANSWER 10 OF 12 MEDLINE on STN

1989313290 PubMed ID: 2546005. Phospholipase C and haemolytic activities of Clostridium perfringens alpha-**toxin** cloned in Escherichia coli: sequence and homology with a Bacillus cereus phospholipase C. Leslie D; Fairweather N; Pickard D; Dougan G; Kehoe M. (Department of Microbiology, University of Newcastle upon Tyne, Medical School, Framlington Place, UK.) Molecular microbiology, **(1989 Mar)** Vol. 3, No. 3, pp. 383-92. Journal code: 8712028. ISSN: 0950-382X. L-ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The Clostridium perfringens alpha-**toxin** (phospholipase C) gene (cpa) has been cloned and expressed in Escherichia coli. The biological activities of the cloned gene product have been analysed and the complete nucleotide sequence of the cpa gene has been determined. The cloned cpa gene product, which is exported to the **periplasm** in **E. coli**, possesses both phospholipase C and haemolytic activities. Haemolysis is not apparent when cell extracts are incubated with isotonic suspensions of sheep erythrocytes, but can be detected and quantified readily when dilutions of the same extracts are placed in wells in sheep-blood agar plates. Like other sequenced clostridial genes, the cpa gene has a high AT content (66.4%), exhibits a strong bias for using codons with A or T in the wobble position, and the 350 base pairs upstream from the gene have a significantly higher AT content (79.5%) than the coding region. The cpa gene encodes a 398 amino acid polypeptide with a deduced molecular weight of 45,481 D. This is very similar to the estimated molecular weight (Mr) of the cpa primary gene product expressed in an in vitro transcription-translation system (Mr 46,000), but larger than the cpa gene product detected in **E. coli** minicells, **E. coli** whole cells or in C. perfringens cells (Mr 43,000), suggesting post-translational processing. The 28 N-terminal residues of the deduced alpha-**toxin** sequence possess the consensus features of a signal peptide and may be removed during secretion. The deduced alpha-**toxin** sequence shares significant structural homology with the phosphatidylcholine-preferring phospholipase C of Bacillus cereus.

L152 ANSWER 11 OF 12 MEDLINE on STN

1989032622 PubMed ID: 2903127. Cloning and expression in Escherichia coli of the perfringolysin O (theta-**toxin**) gene from Clostridium perfringens and characterization of the gene product. Tweten R K. (Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City 73190.) Infection and immunity, **(1988 Dec)** Vol. 56, No. 12, pp. 3228-34. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.
Report No.: NLM-PMC259729. Pub. country: United States. Language: English.

AB The gene encoding perfringolysin O, the thiol-activated hemolysin from Clostridium perfringens (ATCC 13124), was cloned and expressed in Escherichia coli. A gene library of C. perfringens chromosomal DNA was constructed in bacteriophage lambda EMBL3. A recombinant was identified that produced a hemolysin that was inhibited by cholesterol and was tentatively identified as perfringolysin O. Subcloning experiments localized the perfringolysin O gene (pfo) to a 1.8-kilobase region on the cloned chromosomal fragment. **E. coli** which carried a plasmid subclone of pfo (pRT1B) expressed perfringolysin O and secreted it into the **periplasm**. The amino-terminal sequence of the pfo gene product was identical with that determined for perfringolysin O purified from C. perfringens, indicating that **E. coli** correctly removed the signal peptide during secretion. Purification of the pfo product was accomplished by high-resolution gel filtration and

anion-exchange chromatography. Analysis of the pfo product by sodium dodecyl sulfate gel electrophoresis showed that it comigrated with authentic perfringolysin O; both had an estimated molecular weight of 54,000. Two-dimensional tryptic peptide maps of the pfo product and of authentic perfringolysin O purified from C. perfringens were identical. The hemolytic activity of the pfo product was similar to that of authentic perfringolysin O; one hemolytic unit (HU) of the cloned gene product or authentic perfringolysin O corresponded to approximately 1 ng or a hemolytic activity of 10(6) HU per mg.

L152 ANSWER 12 OF 12 MEDLINE on STN DUPLICATE 4
1988297157 PubMed ID: 2841198. Hybrid enterotoxin LTA::STa proteins and their protection from degradation by in vivo association with B-subunits of Escherichia coli heat-labile enterotoxin. Sanchez J; Hirst T R; Uhlin B E. (Department of Medical Microbiology, University of Goteborg, Sweden.) Gene, **(1988 Apr 29)** Vol. 64, No. 2, pp. 265-75. Journal code: 7706761. ISSN: 0378-1119. L-ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB Chimeric proteins exhibiting antigenic determinants of the heat-labile enterotoxin (LT) and heat-stable (STa) enterotoxins on the same molecule may provide a means to obtain immunoprophylactic and diagnostic reagents for Escherichia coli-caused diarrhea. We recently showed that fusion of two different lengths of the STa gene to the C end of the A-subunit of LT (LTA) results in LTA::STa fusion proteins as monitored by GM1-ELISA [Sanchez et al.: FEBS Lett. 208 (1986) 194-198]. Here we determine the approximate molecular size of the LTA::STa fusion proteins and provide further evidence of their hybrid nature by immunoblot analysis. Using this technique we also demonstrate that to obtain detectable amounts of these **recombinant proteins** it is essential to coexpress them with the respective B-subunit of LT (LTB). We propose that this dependence on coexpression reflects the association between the LTA::STa hybrids and LTB subunits. The resulting LTA::STa/LTB complexes were found in the **E. coli periplasm**. This indicated that the exported hybrids, once associated with LTB, were stabilized and formed molecules that behaved essentially as native LT. The protective effect exerted by the B-subunit might conceivably be extended to other LTA-derived hybrid proteins, thus allowing the fusion of other foreign peptides to LTA and their subsequent recovery in the same fashion.

```
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L154         0 L70 AND BEE PROTEASE

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L155         0 L70 AND COCKROADH

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L156         0 L70 AND COCKROACH

=> s l70 and midge
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L158         0 L70 AND HORNET

=> s l70 and phospholipase
L159         3 L70 AND PHOSPHOLIPASE

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L160         0 L59 AND DEAD
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L163 2 DUP REMOVE L162 (0 DUPLICATES REMOVED)

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L163 ANSWER 1 OF 2 MEDLINE on STN
1998129912 PubMed ID: 9468660. Release of miniantibodies from **E. coli** cells into the supernatant at low and high cell densities.
Morbe J L; Riesenberg D. (Institute of Toxicology, ETH, Schwerzenbach, Switzerland.) Microbiological research, **(1997 Dec)** Vol. 152, No. 4, pp. 385-94. Journal code: 9437794. ISSN: 0944-5013. L-ISSN: 0944-5013. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB By chemical permeabilization of **E. coli** cells with detergents and membrane active peptides considerable amounts of the periplasmic anti-PC-miniantibodies can be released. Releases of active miniantibodies of 31%, respectively of 38%, were obtained by exposing low cell density suspensions to nonionic detergents like Triton X-100 and tetraethyleneglycolmonodecylether. At high cell densities releasing levels of 40% and 56% were observed for these detergents. The addition of cationic, membrane active peptides (PMBN, polylysine 115, magainin II and melittin) led to release of up to 20% of active miniantibodies at low cell densities. The excretion of miniantibodies at low cell densities was increased up to 35% by **phospholipase** A2. Interestingly, the membrane associating properties of the anti-PC-miniantibodies influenced the permeability of the outer membrane and the excretion of beta-lactamase.

L163 ANSWER 2 OF 2 MEDLINE on STN
1989313290 PubMed ID: 2546005. **Phospholipase** C and haemolytic activities of Clostridium perfringens alpha-toxin cloned in Escherichia coli: sequence and homology with a Bacillus cereus **phospholipase** C. Leslie D; Fairweather N; Pickard D; Dougan G; Kehoe M. (Department of Microbiology, University of Newcastle upon Tyne, Medical School, Framlington Place, UK.) Molecular microbiology, **(1989 Mar)** Vol. 3, No. 3, pp. 383-92. Journal code: 8712028. ISSN: 0950-382X. L-ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The Clostridium perfringens alpha-toxin (**phospholipase** C) gene (cpa) has been cloned and expressed in Escherichia coli. The biological activities of the cloned gene product have been analysed and the complete nucleotide sequence of the cpa gene has been determined. The cloned cpa gene product, which is exported to the **periplasm** in **E. coli**, possesses both **phospholipase** C and haemolytic activities. Haemolysis is not apparent when cell extracts are incubated with isotonic suspensions of sheep erythrocytes, but can be detected and quantified readily when dilutions of the same extracts are placed in wells in sheep-blood agar plates. Like other sequenced clostridial genes, the cpa gene has a high AT content (66.4%), exhibits a strong bias for using codons with A or T in the wobble position, and the 350 base pairs upstream from the gene have a significantly higher AT content (79.5%) than the coding region. The cpa gene encodes a 398 amino acid polypeptide with a deduced molecular weight of 45,481 D. This is very similar to the estimated molecular weight (Mr) of the cpa primary gene product expressed in an in vitro transcription-translation system (Mr 46,000), but larger than the cpa gene product detected in **E. coli**

minicells, **E. coli** whole cells or in C. perfringens cells (Mr 43,000), suggesting post-translational processing. The 28 N-terminal residues of the deduced alpha-toxin sequence possess the consensus features of a signal peptide and may be removed during secretion. The deduced alpha-toxin sequence shares significant structural homology with the phosphatidylcholine-preferring **phospholipase** C of Bacillus cereus.

=> s l70 and hornet antigen
L164 0 L70 AND HORNET ANTIGEN

=> s l70 and wasp
L165 0 L70 AND WASP

=> s l70 and yellojacket
L166 0 L70 AND YELLOJACKET

=> s l70 and yellowjacket
L167 0 L70 AND YELLOWJACKET

=> s l70 and wasp
L168 0 L70 AND WASP

=> s l70 and ant
L169 1 L70 AND ANT

=> d l169 cbib abs

L169 ANSWER 1 OF 1 MEDLINE on STN
2002355324 PubMed ID: 12098781. Expression of gene encoding GL-7ACA acylase in Escherichia coli. Wang En-Duo; Zheng Yong-Gang; Li Yong; Jiang Wei-Hong; Yang Yun-Liu. (State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200031, China. wed@server.shcnc.ac.cn) . Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica, (2002 Jul) Vol. 34, No. 4, pp. 526-31. Journal code: 20730160R. ISSN: 0582-9879. L-ISSN: 0582-9879. Pub. country: China. Language: English.
AB Glutaryl 7-amino cephalosporanic acid acylase (GL-7ACA acylase) from Pseudomonas sp.130 catalyzes hydrolysis of glutaryl 7-amino cephalosporanic acid to produce 7-amino cephalosporanic acid (7-ACA). 7-ACA is the starting material for the industrial production of most cephalosparonic derivatives. Six plasmids for expression of GL-7ACA acylase were constructed and these recombin **ant** plasmids presented different expression characteristics in Escherichia coli. The acylase gene from plasmid pKKCA1 was inserted into plasmid pMFT7-5 and the resulting plasmid pMFT7CA1 has higher expression in **E. coli.** The specific activity of the crude extract of the transformant JM109(DE3)/pMFT7CA1 was near 5 u/g, so the over produced enzyme was easily purified by a single-step anion exchange column chromatography. The enzyme could be purified by immobilized ion affinity chromatography after fused by 6xHis in the N-terminal of its alpha-subunit. Because plasmid pSMLCA1 brings tc(R) and p15A origin, it is special useful plasmid in fermentation. Two secretory expression plasmids, pSUCA1S and pETCA1pelB, could secrete the acylase to periplasmic space of bacteria. The whole cells containing the secretory expression plasmid may be used for production of 7-ACA directly.

=> s l70 and fire ant
L170 0 L70 AND FIRE ANT

```
=> s l70 and hyaluronidase
L171         0 L70 AND HYALURONIDASE

=> s l70 and cod
L172         0 L70 AND COD

=> s l70 and parvalbumin
L173         0 L70 AND PARVALBUMIN

=> s l70 and salmon
L174         0 L70 AND SALMON

=> s l70 and casein
L175         6 L70 AND CASEIN

=> dup remove l175
PROCESSING COMPLETED FOR L175
L176          2 DUP REMOVE L175 (4 DUPLICATES REMOVED)

=> d l716 and pd<20000406
L716 NOT FOUND
The L-number entered has not been defined in this session, or it
has been deleted.  To see the L-numbers currently defined in this
session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> d l176 1-2 cbib abs

L176 ANSWER 1 OF 2     MEDLINE on STN                    DUPLICATE 1
2008093546   PubMed ID: 18189353.   Preparation of unglycosylated human
     caseinomacropeptide by engineering DAB Escherichia coli. Liu Fangjie; Liao
     Liang; Chen Jinchun. (College of Life Science and Technology, Beijing
     University of Chemical Technology, Beijing 100029, People's Republic of
     China. ) Journal of agricultural and food chemistry, (2008 Feb 13) Vol.
     56, No. 3, pp. 889-93.  Electronic Publication: 2008-01-12. Journal code:
     0374755. ISSN: 0021-8561. L-ISSN: 0021-8561. Pub. country: United States.
     Language: English.
AB   Human caseinomacropeptide (hCMP) is 65 amino acids in length and was
     originally derived from the C terminus of human milk kappa-casein
     .  As it is highly abundant in both essential amino acids and branched
     amino acids, it could be developed as a practical food and even as
     medicinal nutrition for patients.  This study was undertaken to prepare
     recombinant hCMP without glycosylation using recombinant plasmid and
     prokaryotic expression system.  The gene encoding hCMP was chemically
     synthesized and directly inserted into the pET28a(+) vector and then
     expressed in Escherichia coli BL21(DE3).  The maximum amount of soluble
     protein was obtained by incubation with 0.5 mM
     isopropyl-alpha-D-thiogalactopyranoside at 30 degrees C for 4 h and
     accounted for 40% of the total intracellular protein.  Most of the
     expressed fusion proteins, located in the cell periplasm and
     cytoplasm, could be adsorbed by nickel affinity chromatography and eluted
     with buffer containing 300 mM imidazole.  The fusion proteins were cleaved
     by enterokinase to remove the 6-His tag.  Gel filtration chromatography
     with Sephadex G-10 was performed for desalting and purification.  A final
     yield of 25 mg of the mature protein with high purity up to 99% was
     obtained from 1 L of E. coli culture.  The purified
     protein was confirmed by MALDI-TOF-MS analysis.  This study overcame the
     problem of glycosylation in hCMP and established a novel approach for the
     preparation of unglycosylated hCMP.

L176 ANSWER 2 OF 2     MEDLINE on STN                    DUPLICATE 2
1991144561   PubMed ID: 1996969.   Metalloendopeptidase QG. Isolation from
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Escherichia coli and characterization. Polgar L; Szigetvari A; Low M; Korodi I; Balla E. (Institute of Enzymology Biological Research Center, Hungarian Academy of Sciences, Budapest.) The Biochemical journal, (1991 Feb 1) Vol. 273 (Pt 3), pp. 725-31. Journal code: 2984726R. ISSN: 0264-6021. L-ISSN: 0264-6021.
Report No.: NLM-PMC1149823. Pub. country: ENGLAND: United Kingdom.
Language: English.

AB A new proteinase, which preferentially cleaves the Gln-Gly bond, was isolated from Escherichia coli. Because of this narrow specificity, the enzyme was called metalloendopeptidase QG. The proteinase is a monomer and consists of a single polypeptide chain of Mr 67,000, which is significantly smaller than the other known metalloendopeptidases of **E. coli**. It is found in the cytoplasm, but not in the **periplasm**. The enzyme cleaves the substrate benzyloxycarbonyl-Gln-Gly-Pro 2-naphthylamide between the glutamine and glycine residues, as well as its extended homologues including a nonapeptide, but it does not hydrolyse either the oxidized A and B chains of insulin or azo-**casein**. The pH-dependence of substrate hydrolysis gives a bell-shaped curve with pK1 = 6.6 and pK2 = 8.8. The metallopeptidase is inhibited in Tris and imidazole buffers, the basic components of which are presumably liganded to the essential Zn2+ ion. 2-Aminobenzoyl-Gln-Gly-Pro 2-naphthylamide, designed as a fluorescent substrate for the metallopeptidase, proved to be a strong inhibitor. Bestatin, an inhibitor of aminopeptidases in the micromolar concentration range, inhibits the metalloendopeptidase only in the millimolar concentration range. Captopril, the widely used inhibitor of angiotensin-converting enzyme, is a fairly good inhibitor of the metalloendopeptidase. The simplest inhibitor that can be used to protect **recombinant proteins** from degradation by the metalloendopeptidase may be EDTA, which is effective at low millimolar concentration.

=> d his

(FILE 'HOME' ENTERED AT 13:33:55 ON 23 FEB 2011)

FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 13:34:18 ON 23 FEB 2011

L1	1554	S	MUCOSAL DELIVERY
L2	29	S	L1 AND E COLI
L3	0	S	L2 AND RECOMBINANT ALLERGEN
L4	0	S	L2 AND DEAD
L5	0	S	L2 AND KILLED
L6	0	S	L2 AND ALLERGEN
L7	4235130	S	COMPOSITION
L8	4961	S	L7 AND RECTAL
L9	26	S	L8 AND E COLI
L10	0	S	L9 AND ENCAPSULATED ALLERGEN
L11	1	S	L9 AND ALLERGEN
L12	0	S	L11 AND DEAD
L13	4260	S	L7 AND VAGINAL
L14	25	S	L13 AND E COLI
L15	0	S	L14 AND ALLERGEN
L16	0	S	L14 AND ENCAPSULATED
L17	0	S	L14 AND DEAD
L18	0	S	L17 AND NASAL
L19	6906	S	L7 AND NASAL
L20	31	S	L19 AND E COLI
L21	1	S	L20 AND ALLERGEN
L22	0	S	L21 AND DEAD
L23	0	S	L21 AND KILLED

L24 63559 S L7 AND ORAL
L25 200 S L24 AND E COLI
L26 1 S L25 AND ALLERGEN
L27 0 S L26 AND DEAD
L28 2 S L25 AND ENCAPSULATED
L29 2 DUP REMOVE L28 (0 DUPLICATES REMOVED)
L30 2920 S L7 AND BUCCAL
L31 5 S L30 AND E COLI
L32 0 S L31 AND ALLERGEN
L33 0 S L31 AND OVALBUMIN
L34 0 S L31 AND DEAD
L35 814139 S VACCINE
L36 11437 S L35 AND E COLI
L37 14 S L36 AND RECOMBINANT ALLERGEN
L38 0 S L37 AND PERIPLASM
L39 0 S L37 AND NON-SECRETED
L40 0 S L37 AND DEAD
L41 0 S L37 AND HEAT KILLED
L42 409 S VACCINE DELIVERY VEHICLE
L43 19 S L42 AND E COLI
L44 0 S L43 AND ALLERGEN
L45 0 S L43 AND DEAD
L46 73061 S ANTIGEN PRESENTATION
L47 0 S L46 AND DEAD E COLI
L48 382 S L46 AND E COLI
L49 3 S L48 AND RECOMBINANT ALLERGEN
L50 0 S L49 AND KILLED
L51 0 S L49 AND DEAD
L52 2 DUP REMOVE L49 (1 DUPLICATE REMOVED)
L53 3646 S RECOMBINANT ALLERGEN
L54 86 S L53 AND RAGWEED
L55 1 S L54 AND E COLI
L56 0 S L55 AND DEAD
L57 0 S L55 AND NONSECRETED
L58 0 S L55 AND PERIPLASM
L59 61 S L53 AND MUGWORT
L60 0 S L59 AND E COLI
L61 17 S L53 AND SUNFLOWER
L62 6 S L61 AND E COLI
L63 0 S L62 AND PERIPLASM
L64 0 S L62 AND NON-SECRETED
L65 0 S L62 AND DEAD
L66 2 DUP REMOVE L62 (4 DUPLICATES REMOVED)
L67 522403 S E COLI
L68 26748 S L67 AND RECOMBINANT PROTEIN
L69 0 S L68 AND NON-SECRETED
L70 673 S L68 AND PERIPLASM
L71 5 S L70 AND ALLERGEN
L72 2 DUP REMOVE L71 (3 DUPLICATES REMOVED)
L73 0 S L70 AND OVALBUMIN
L74 3 S L70 AND GRASS
L75 3 S L74 AND PERIPLASM
L76 1 DUP REMOVE L75 (2 DUPLICATES REMOVED)
L77 0 S L68 AND STEALTH DELIVERY
L78 19 S L68 AND ANTIGEN PRESENTING CELL
L79 0 S L78 AND NONSECRETED
L80 13 DUP REMOVE L78 (6 DUPLICATES REMOVED)
L81 8 S L80 AND PD<20000406
L82 8 DUP REMOVE L81 (0 DUPLICATES REMOVED)
L83 0 S L70 AND MUGWORT
L84 0 S L70 AND SUNFLOWER PROFILIN
L85 0 S L70 AND ANNUAL MERCURY PROFILIN

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L86           0 S L70 AND "AMB A 1"
L87           0 S L70 AND BERMUDA GRASS
L88           0 S L70 AND ORCHARD GRASS
L89           0 S L70 AND VELVET GRASS
L90           3 S L70 AND GRASS
L91           0 S L90 AND DEAD
L92           1 DUP REMOVE L90 (2 DUPLICATES REMOVED)
L93           0 S L70 AND CANARY GRASS
L94           0 S L70 AND TIMOTHY GRASS
L95           0 S L70 AND KENTUCKY BLUE GRASS
L96           0 S L70 AND JOHNSON GRASS
L97           0 S L70 AND ALDER
L98           2 S L70 AND BIRCH
L99           1 DUP REMOVE L98 (1 DUPLICATE REMOVED)
L100          0 S L70 AND HORNBEAM
L101          0 S L70 AND CHESTNUT
L102          0 S L70 AND HAZEL
L103          0 S L70 AND OAK
L104          0 S L70 AND JAPONICA
L105          0 S L70 AND CEDAR
L106          0 S L70 AND ASH
L107          0 S L70 AND OLIVE
L108          0 S L70 AND LILAC
L109          0 S L70 AND MITE
L110          0 S L70 AND "DER P1"
L111        522 S "DER P1"
L112          1 S L111 AND E COLI
L113          0 S L70 AND PTERONYSSINUS
L114          0 S L70 AND COW LIPOCALIN
L115          1 S L70 AND ALPHA-LACTALBUMIN
L116          0 S L70 AND SERUM ALBULIN
L117          0 S L70 AND COW SERUM ALBUMIN
L118          0 S L70 AND ALBUMIN
L119      29580 S 70 AND IMMUNOGLOBULIN
L120          9 S L119 AND PERIPLASM
L121          4 DUP REMOVE L120 (5 DUPLICATES REMOVED)
L122          6 S L70 AND CASEIN
L123          2 DUP REMOVE L122 (4 DUPLICATES REMOVED)
L124          0 S L70 AND ALBUMIN
L125         15 S L70 AND LIPOCALIN
L126          3 S L125 AND PD<20000406
L127          3 DUP REMOVE L126 (0 DUPLICATES REMOVED)
L128          0 S L70 AND "FEL D1"
L129          0 S L70 AND CAT ANTIGEN
L130          0 S L70 AND MUP ANTIGEN
L131          0 S L70 AND FUNGUS ANTIGEN
L132          0 S L70 AND HEAT SHOCL PROTEIN
L133          0 S L70 AND YCP4
L134          0 S L70 AND C HERBARUM
L135          0 S L70 AND FUGUS
L136          2 S L70 AND FUNGUS
L137          2 DUP REMOVE L136 (0 DUPLICATES REMOVED)
L138          1 S L137 AND PD<20000406
L139          0 S L70 AND SHAGGY CAP
L140          0 S L70 AND COPRINUS COMATUS
L141          0 S L70 AND APYRASE
L142          0 S L41 AND MOSQUITO
L143          0 S L70 AND AED
L144          0 S L70 AND HONEY BEE
L145          3 S L70 AND PHOSPHOLIPASE
L146          0 S L145 AND BEE
L147          3 DUP REMOVE L145 (0 DUPLICATES REMOVED)
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L148         40 S L70 AND TOXIN
L149          0 S L48 AND NON-SECRETED
L150          0 S L148 AND ENCAPSULATED
L151         22 S L148 AND PD<20000406
L152         12 DUP REMOVE L151 (10 DUPLICATES REMOVED)
L153          0 S L152 AND DEAD
L154          0 S L70 AND BEE PROTEASE
L155          0 S L70 AND COCKROADH
L156          0 S L70 AND COCKROACH
L157          0 S L70 AND MIDGE
L158          0 S L70 AND HORNET
L159          3 S L70 AND PHOSPHOLIPASE
L160          0 S L59 AND DEAD
L161          0 S L159 AND NON-SECRETED
L162          2 S L159 AND PD<20000406
L163          2 DUP REMOVE L162 (0 DUPLICATES REMOVED)
L164          0 S L70 AND HORNET ANTIGEN
L165          0 S L70 AND WASP
L166          0 S L70 AND YELLOJACKET
L167          0 S L70 AND YELLOWJACKET
L168          0 S L70 AND WASP
L169          1 S L70 AND ANT
L170          0 S L70 AND FIRE ANT
L171          0 S L70 AND HYALURONIDASE
L172          0 S L70 AND COD
L173          0 S L70 AND PARVALBUMIN
L174          0 S L70 AND SALMON
L175          6 S L70 AND CASEIN
L176          2 DUP REMOVE L175 (4 DUPLICATES REMOVED)

=> s l70 and  ovomucoid
L177         0 L70 AND OVOMUCOID

=> s l70 and chicken
L178         2 L70 AND CHICKEN

=> dup remove l178
PROCESSING COMPLETED FOR L178
L179          2 DUP REMOVE L178 (0 DUPLICATES REMOVED)

=> d l179 1-2 cbib abs

L179 ANSWER 1 OF 2     MEDLINE on STN
1993252782   PubMed ID: 8098033.   Escherichia coli thioesterase I, molecular
     cloning and sequencing of the structural gene and identification as a
     periplasmic enzyme. Cho H; Cronan J E Jr. (Department of Microbiology,
     University of Illinois, Urbana-Champaign 61801. ) The Journal of
     biological chemistry, (1993 May 5) Vol. 268, No. 13, pp. 9238-45.  Journal
     code: 2985121R. ISSN: 0021-9258. L-ISSN: 0021-9258. Pub. country: United
     States. Language: English.
AB   The structural gene for Escherichia coli thioesterase I (called tesA) has
     been cloned by use of sequence data obtained from the purified protein.
     The tesA gene was mapped at 530 kilobase pair of the E.
     coli physical map (minute 11.6 of E. coli
     genetic map).  The DNA sequence of the tesA gene was obtained and the
     deduced protein sequence showed that thioesterase I consists of 182 amino
     acids and has a molecular mass of 20.5 kDa.  Comparison of the DNA and
     protein sequence data suggested that a leader sequence of 26 amino acid
     residues is cleaved from the primary translation product, and this
     processing was confirmed by NH2-terminal sequencing of the primary
     translation product synthesized in vitro.  These data predicted that
     thioesterase I (long believed to be a cytoplasmic protein) is exported to
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the cell **periplasm**, a prediction supported by release of the enzyme from cells upon osmotic shock. The TesA protein sequence does not exhibit any significant overall sequence similarity with other known proteins, although the sequence does contain two small sequence elements found in several other thioesterases. One of these elements is a sequence similar to the serine esterase active sites found in serine proteases and four other thioesterases. A serine residue within this TesA element was shown to be covalently labeled with [3H] diisopropyl fluorophosphate, a potent inhibitor of TesA activity. The second sequence element is a histidine-containing sequence found close to the carboxyl terminus that is also found in the carboxyl termini of the four known active serine thioesterases. The physiological role of thioesterase I is unclear. A strain carrying a null mutation of the tesA gene was constructed and found to have no growth phenotype. Moreover, a strain carrying a plasmid that gave massive overproduction of TesA (approximately 100-fold higher than that of the wild type) also grew normally. In addition a strain containing double null mutations in both tesA and tesB (the structural gene for **E. coli** thioesterase II) also failed to display any growth phenotype. Analysis of the fatty acid compositions of phospholipid, lipid A, and lipoprotein of the above strains showed no significant changes from a wild type strain.

L179 ANSWER 2 OF 2 MEDLINE on STN

1991348031 PubMed ID: 1879418. Purification and characterization of a **chicken** egg white cystatin variant expressed in an Escherichia coli pIN-III-ompA system. Auerswald E A; Genenger G; Mentele R; Lenzen S; Assfalg-Machleidt I; Mitschang L; Oschkinat H; Fritz H. (Abteilung fur Klinische Chemie und Klinische Biochemie, Chirurgischen Klinik Innenstadt, Universitat Munchen, Federal Republic of Germany.) European journal of biochemistry / FEBS, (1991 Aug 15) Vol. 200, No. 1, pp. 131-8. Journal code: 0107600. ISSN: 0014-2956. L-ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A synthetic gene coding for a **chicken** egg white cystatin variant was cloned and expressed using the pIN-III-ompA Escherichia coli expression system. After osmotic shock of the **E. coli** cells, the cysteine proteinase inhibitor was isolated from **periplasm** and purified by S-carboxymethylpapain affinity chromatography. The resulting inhibitory material was characterized by SDS/PAGE, reversed-phase HPLC, peptide mapping and amino acid sequencing. The recombinant variant **chicken** AEF-[S1----M, M29----I, M89----L]cystatin shows strong inhibitory activity and displays Ki values in the complex with papain, actinidin and cathepsin B similar to those found for natural **chicken** cystatin. The purified variant showed a native-**chicken**-cystatin-like conformational state, as determined by NMR spectroscopy, if the NMR data of 15N-labelled recombinant inhibitor were compared with those of the natural inhibitor.

=> s l70 and shrimp
MAXIMUM L# WOULD BE EXCEEDED.

=>

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=> LOG Y

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NEWS 14	DEC 21	CAS Learning Solutions -- a new online training experience
NEWS 15	DEC 22	Value-Added Indexing Improves Access to World Traditional Medicine Patents in CAplus
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NEWS 17	JAN 26	Improved Timeliness of CAS Indexing Adds Value to USPATFULL and USPAT2 Chemistry Patents
NEWS 18	JAN 26	Updated MeSH vocabulary, new structured abstracts, and other enhancements improve searching in STN reload of MEDLINE

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                                                         ENTRY    SESSION
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=> s recombinant protein
L1      284807 RECOMBINANT PROTEIN

=> s l1 and E coli
L2       26748 L1 AND E COLI

=> s l2 and encapsulated
L3          24 L2 AND ENCAPSULATED

=> s l3 and pd<20000406
L4           4 L3 AND PD<20000406

=> dup remove l4
PROCESSING COMPLETED FOR L4
L5           3 DUP REMOVE L4 (1 DUPLICATE REMOVED)
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=> d l5 1-3 cbib abs

L5 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
1998147697 PubMed ID: 9488373. The Haemophilus influenzae HtrA protein is a protective antigen. Loosmore S M; Yang Y P; Oomen R; Shortreed J M; Coleman D C; Klein M H. (Pasteur Merieux Connaught Canada Research, North York, Ontario. sloosmore@ca.pmc-vacc.com) . Infection and immunity, **(1998 Mar)** Vol. 66, No. 3, pp. 899-906. Journal code: 0246127. ISSN: 0019-9567. L-ISSN: 0019-9567.
Report No.: NLM-PMC107993. Pub. country: United States. Language: English.
AB The htrA gene from two strains of nontypeable Haemophilus influenzae has been cloned and sequenced, and the encoded approximately 46-kDa HtrA proteins were found to be highly conserved. H. influenzae HtrA has approximately 55% identity with the Escherichia coli and Salmonella typhimurium HtrA stress response proteins, and expression of the H. influenzae htrA gene was inducible by high temperature. Recombinant HtrA (rHtrA) was expressed from **E. coli**, and the purified protein was found to have serine protease activity. rHtrA was found to be very immunogenic and partially protective in both the passive infant rat model of bacteremia and the active chinchilla model of otitis media. Immunoblot analysis indicated that HtrA is antigenically conserved in **encapsulated** and nontypeable H. influenzae species. Site-directed mutagenesis was performed on the htrA gene to ablate the endogenous serine protease activity of wild-type HtrA, and it was found that eight of nine recombinant mutant proteins had no measurable residual proteolytic activity. Two mutant proteins were tested in the animal protection models, and one, H91A, was found to be partially protective in both models. H91A HtrA may be a good candidate antigen for a vaccine against invasive H. influenzae type b disease and otitis media and is currently in phase I clinical trials.

L5 ANSWER 2 OF 3 MEDLINE on STN
1997000206 PubMed ID: 8843348. Development of a metallothionein based heavy metal biosorbent. Pazirandeh M. (Center for Biomolecular Science and Engineering, Naval Research Laboratory, Washington, DC 20375, USA.) Biochemistry and molecular biology international, **(1996 Jul)** Vol. 39, No. 4, pp. 789-95. Journal code: 9306673. ISSN: 1039-9712. L-ISSN: 1039-9712. Pub. country: Australia. Language: English.
AB The potential utility of a recombinant **E. coli** expressing the Neurospora crassa metallothionein gene (NCP) as a heavy metal biosorbent was investigated. It was shown that the NCP was capable of efficiently removing low levels of several metals (including cadmium, lead, and mercury) from solutions. The reusability of the NCP was demonstrated through 5 cycles of metal binding, stripping with dilute acid, and regeneration of the binding sites with out any adverse effect on the metal binding activity. The NCP was successfully **encapsulated** in alginate and acrylamide without any inhibitory effect on its metal uptake activity. Furthermore, the metal uptake activity of the NCP was shown to be metabolism independent and resistant to solvents and other compounds (eg. polyaromatic hydrocarbons) which are often present along with heavy metals in waste waters thereby creating the potential for non-viable, **encapsulated** cells to be used.

L5 ANSWER 3 OF 3 MEDLINE on STN
1992395133 PubMed ID: 1522221. Human bactericidal/permeability-increasing protein and a recombinant NH2-terminal fragment cause killing of serum-resistant gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria. Weiss J; Elsbach P; Shu C; Castillo J; Grinna L; Horwitz A; Theofan G. (Department of Microbiology, New York University School of Medicine, New York 10016.) The Journal of clinical investigation, **(1992 Sep)** Vol. 90, No. 3, pp. 1122-30. Journal code: 7802877. ISSN: 0021-9738. L-ISSN:

0021-9738.
Report No.: NLM-PMC329974. Pub. country: United States. Language: English.

AB The bactericidal/permeability-increasing protein (BPI) of neutrophils and BPI fragments neutralize the effects of isolated Gram-negative bacterial lipopolysaccharides both in vitro and in vivo. Since endotoxin most commonly enters the host as constituents of invading Gram-negative bacteria, we raised the question: Can BPI and its bioactive fragments also protect against whole bacteria? To determine whether the bactericidal and endotoxin-neutralizing activities of BPI/fragments are expressed when Gram-negative bacteria are introduced to the complex environment of whole blood we examined the effects of added BPI and proteolytically prepared and recombinant NH2-terminal fragments on: (a) the fate of serum-resistant **encapsulated** Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa that survive the antibacterial actions of whole blood and (b) the ability of these bacteria to trigger cytokine release. Added BPI in nanomolar concentrations killed each of three **encapsulated** strains of **E. coli** and in closely parallel fashion inhibited tumor necrosis factor (TNF) release. Holo-BPI and its NH2-terminal fragment were equipotent toward a rough LPS chemotype K1-**encapsulated** strain, but the fragment was substantially more potent than holo-BPI toward two **encapsulated** smooth LPS chemotype strains. TNF release induced by K. pneumoniae and P. aeruginosa was also inhibited by both holo-BPI and fragment but, at the protein concentrations tested, P. aeruginosa was killed only by the fragment and K. pneumoniae was not killed by either protein. The bactericidal action of BPI/fragment toward **E. coli** is inhibited by C7-depleted serum, but accelerated by normal serum, indicating that BPI, acting in synergy with late complement components, enhances extracellular killing of serum-resistant bacteria. Thus, BPI and an even more potent NH2-terminal fragment may protect against Gram-negative bacteria in the host by blocking bacterial proliferation as well as endotoxin-mediated effects, not only as components of the intracellular antibacterial arsenal of the neutrophil, but also as potentially therapeutic extracellular agents.

```
=> s l2 and periplasm
L6         673 L2 AND PERIPLASM

=> s l6 and "not secreted"
L7           0 L6 AND "NOT SECRETED"

=> s l6 and "non-secrete"
L8           0 L6 AND "NON-SECRETE"

=> s l6 and tropomyosin
L9           0 L6 AND TROPOMYOSIN

=> s l6 and shrimp
L10          0 L6 AND SHRIMP

=> s l6 and abalone
L11          0 L6 AND ABALONE

=> s l6 and squid
L12          1 L6 AND SQUID

=> d l12 cbib abs

L12  ANSWER 1 OF 1     MEDLINE on STN
1998215175   PubMed ID: 9555890.   The periplasmic, group III catalase of
     Vibrio fischeri is required for normal symbiotic competence and is induced
```

both by oxidative stress and by approach to stationary phase. Visick K L; Ruby E G. (Pacific Biomedical Research Center, University of Hawaii, Honolulu 96813, USA.) Journal of bacteriology, (1998 Apr) Vol. 180, No. 8, pp. 2087-92. Journal code: 2985120R. ISSN: 0021-9193. L-ISSN: 0021-9193.
Report No.: NLM-PMC107134. Pub. country: United States. Language: English.

AB The catalase gene, katA, of the sepiolid **squid** symbiont Vibrio fischeri has been cloned and sequenced. The predicted amino acid sequence of KatA has a high degree of similarity to the recently defined group III catalases, including those found in Haemophilus influenzae, Bacteroides fragilis, and Proteus mirabilis. Upstream of the predicted start codon of katA is a sequence that closely matches the consensus sequence for promoters regulated in Escherichia coli by the alternative sigma factor encoded by rpoS. Further, the level of expression of the cloned katA gene in an **E. coli** rpoS mutant is much lower than in wild-type **E. coli**. Catalase activity is induced three- to fourfold both as growing V. fischeri cells approach stationary phase and upon the addition of a small amount of hydrogen peroxide during logarithmic growth. The catalase activity was localized in the **periplasm** of wild-type V. fischeri cells, where its role could be to detoxify hydrogen peroxide coming from the external environment. No significant catalase activity could be detected in a katA null mutant strain, demonstrating that KatA is the predominately expressed catalase in V. fischeri and indicating that V. fischeri carries only a single catalase gene. The catalase mutant was defective in its ability to competitively colonize the light organs of juvenile **squids** in coinoculation experiments with the parent strain, suggesting that the catalase enzyme plays an important role in the symbiosis between V. fischeri and its **squid** host.

```
=> s l6 and celery
L13          0 L6 AND CELERY

=> s l6 and mustard
L14          0 L6 AND MUSTARD

=> s l6 and turnip
L15          0 L6 AND TURNIP

=> s l6 and barley
L16          0 L6 AND BARLEY

=> s l6 and corn
L17          3 L6 AND CORN

=> dup remove l17
PROCESSING COMPLETED FOR L17
L18           2 DUP REMOVE L17 (1 DUPLICATE REMOVED)

=> s l18 and pd<20000406
L19          1 L18 AND PD<20000406

=> d l19 cbib abs

L19  ANSWER 1 OF 1     MEDLINE on STN
1996405955   PubMed ID: 8810080.   Cloning and characterization of a xylanase
     gene from corn strains of Erwinia chrysanthemi. Keen N T; Boyd
     C; Henrissat B. (Department of Plant Pathology, University of California,
     Riverside 92521, USA. Keen@ucrac1.ucr.edu) . Molecular plant-microbe
     interactions : MPMI, (1996 Sep) Vol. 9, No. 7, pp. 651-7.
     Journal code: 9107902. ISSN: 0894-0282. L-ISSN: 0894-0282. Pub. country:
```

United States. Language: English.
AB The gene encoding a 42-kDa endoxylanase was cloned from Erwinia chrysanthemi strain D1. Sequencing of this gene, called xynA, showed that it encoded a primary protein product of 413 amino acids with an unusual and long (31 amino acid) leader peptide that was cleaved during secretion to the bacterial **periplasm**. This protein is distinct from xylanases in glycohydrolase families 10 and 11 and, instead, appears to be intermediate between families 5 and 30. The xynA gene is located downstream from a gene with high homology to ATP-dependent RNA helicases and the Escherichia coli recD gene. Large amounts of the mature xylanase were produced by **E. coli** cells carrying a T7 expression plasmid construct and the protein was isolated from the bacterial periplasmic fraction by chromatography on a CM Bio-gel column. Marker exchange mutagenesis of the xynA gene eliminated the ability of strain D1 to produce detectable extracellular xylanase activity but did not affect virulence on **corn** leaves.

```
=> s l6 and hazelnut
L20          0 L6 AND HAZELNUT

=> s l6 and "Bet v1"
L21          0 L6 AND "BET V1"

=> d his

     (FILE 'HOME' ENTERED AT 14:24:50 ON 23 FEB 2011)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 14:25:11 ON
     23 FEB 2011
L1       284807 S RECOMBINANT PROTEIN
L2        26748 S L1 AND E COLI
L3           24 S L2 AND ENCAPSULATED
L4            4 S L3 AND PD<20000406
L5            3 DUP REMOVE L4 (1 DUPLICATE REMOVED)
L6          673 S L2 AND PERIPLASM
L7            0 S L6 AND "NOT SECRETED"
L8            0 S L6 AND "NON-SECRETE"
L9            0 S L6 AND TROPOMYOSIN
L10           0 S L6 AND SHRIMP
L11           0 S L6 AND ABALONE
L12           1 S L6 AND SQUID
L13           0 S L6 AND CELERY
L14           0 S L6 AND MUSTARD
L15           0 S L6 AND TURNIP
L16           0 S L6 AND BARLEY
L17           3 S L6 AND CORN
L18           2 DUP REMOVE L17 (1 DUPLICATE REMOVED)
L19           1 S L18 AND PD<20000406
L20           0 S L6 AND HAZELNUT
L21           0 S L6 AND "BET V1"

=> s l6 and apple
L22          0 L6 AND APPLE

=> s l6 and "Mal d1"
L23          0 L6 AND "MAL D1"

=> s l6 and pear
L24          0 L6 AND PEAR

=> s l6 and  rice
```

L25 0 L6 AND RICE

=> s l6 and ory
L26 0 L6 AND ORY

=> s l6 and ovaocado
L27 0 L6 AND OVAOCADO

=> s l6 and avocado
L28 0 L6 AND AVOCADO

=> s l6 and apricot
L29 0 L6 AND APRICOT

=> s l6 and sweet cherry
L30 2 L6 AND SWEET CHERRY

=> dup remove l30
PROCESSING COMPLETED FOR L30
L31 1 DUP REMOVE L30 (1 DUPLICATE REMOVED)

=> d l31 cbib abs

L31 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
2006113297 PubMed ID: 16499648. Natural and recombinant molecules of the cherry allergen Pru av 2 show diverse structural and B cell characteristics but similar T cell reactivity. Fuchs H C; Bohle B; Dall'Antonia Y; Radauer C; Hoffmann-Sommergruber K; Mari A; Scheiner O; Keller W; Breiteneder H. (Center of Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Mar) Vol. 36, No. 3, pp. 359-68. Journal code: 8906443. ISSN: 0954-7894. L-ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Cherry allergy is often reported in the context of allergy to other fruits of the Rosaceae family and pollinosis to trees because of cross-reactive allergens. Allergic reactions to cherry are reported by 19-29% of birch pollen-allergic patients. Pru av 2, identified as a thaumatin-like protein (TLP) from **sweet cherry**, was recognized by the majority of cherry-allergic patients in immunoblotting.

OBJECTIVES: In order to investigate the structural characteristics and the immunoglobulin (Ig)E- and T cell reactivity of cherry-derived TLP, recombinant Pru av 2 was expressed in Escherichia coli and natural Pru av 2 was purified.

METHODS: Parallel-His and FLAG expression vectors were used for recombinant production of Pru av 2 in the cytoplasm and the **periplasm** of **E. coli**. Natural Pru av 2 was purified from fresh cherries and verified by N-terminal sequencing. Structural characterization was performed using circular dichroism (CD) measurements, and the biologic activity was measured in a glucanase assay. Using cherry-specific sera, the IgE-binding ability of recombinant and natural Pru av 2 was investigated in IgE-ELISA and the T cell reactivity was studied in proliferation assays. Results Natural Pru av 2 revealed thaumatin-like structural features and bound IgE of 50% of cherry-allergic patients. It was demonstrated to be enzymatically active. Recombinant Pru av 2 expressed in the cytoplasm of **E. coli** exhibited a slightly different folding compared with the natural protein. It was not recognized by IgE from cherry-allergic subjects, but retained the ability to stimulate T lymphocytes. Periplasmic recombinant Pru av 2 was able to bind an anti-grape TLP antibody and cherry-specific IgE.

CONCLUSIONS: We prepared two recombinant model TLPs from cherry, and compared their molecular characteristics as well as their IgE-binding activity and T cell interactions in relation to the natural counterpart. The cytoplasmic recombinant Pru av 2 can be used as a hypoallergenic variant in allergen-specific immunotherapy, whereas the periplasmic protein can be included in a component-resolved diagnosis.

```
=> s l6 and peach
L32          0 L6 AND PEACH

=> s l6 and yellow mustard
L33          0 L6 AND YELLOW MUSTARD

=> s l6 and soybean
L34          0 L6 AND SOYBEAN

=> s l6 and peanut
L35          0 L6 AND PEANUT

=> s l6 and Ara h1
L36          0 L6 AND ARA H1

=> s l6 and conglutin
L37          0 L6 AND CONGLUTIN

=> s l6 and "Ara h3"
L38          0 L6 AND "ARA H3"

=> s l6 and "Ara h5"
L39          0 L6 AND "ARA H5"

=> s "Ara h5"
L40          3 "ARA H5"

=> dup remove l40
PROCESSING COMPLETED FOR L40
L41           3 DUP REMOVE L40 (0 DUPLICATES REMOVED)

=> d l41 1-3 cbib abs

L41  ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2011 ACS on STN
2005:1154782   Document No. 143:420855  Microarray immunoassay for mapping
     allergen epitope profile, diagnosing the severity of allergy and
     identifying candidates for anti-IgE antibody therapy.  Sampson, Hugh A.;
     Shreffler, Wayne G; Beyer, Kirsten (Mount Sinai School of Medicine, USA).
     PCT Int. Appl. WO 2005100995 A2 20051027, 80 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO,
     CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR,
     HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
     MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO,
     RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US,
     UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY,
     DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT,
     SE, SN, TD, TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO
     2005-US9567 20050322.  PRIORITY: US 2004-559825P 20040406; US 2004-620923P
     20041021.
AB   The present invention is directed to materials and methods that may be
     used in diagnosing and/or characterizing allergies.  More specifically,
     the specification describes methods and compns. for making and using a
     plurality of peptides having allergen epitopes that may be used in
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immunoassays e.g., microarray based immunoassays to predict the severity of an allergic response.

L41 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2011 ACS on STN
2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen Ara h2 and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein production Alternatively, peanut plants are transformed with peanut allergen antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the Ara h2 gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein production in peanut plants. A full length genomic clone for allergen Ara h2 is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are observed A promoter region was revealed containing a putative TATA box located at position -72. Homologous regions were identified between Ara h2, h6, and h7, and between Ara h3 and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all peanut allergen genes and for down-regulation and silencing of multiple peanut allergen genes.

L41 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2011 ACS on STN
2001:320970 Document No. 135:225750 Four novel recombinant peanut allergens: more information, more problems. Becker, W. -M.; Kleber-Janke, T.; Lepp, U. (Forschungszentrum Borstel, Borstel, D-23845, Germany). International Archives of Allergy and Immunology, 124(1-3), 100-102 (English) 2001. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.

AB An ideal method to clone the allergenic entities completely in peanuts is the phage display system where patients' IgE is the selection and enrichment agent. In this system, the presented allergen and its gene-containing phages are selected by patients' IgE using the panning method. The selected phages are multiplied in Escherichia coli and in the next cycle selected and enriched and so on. After five cycles, six different allergens were cloned. Two of them were the well-known major allergens of peanut, Ara h 1 and Ara h 2. Ara h 4, Ara h 5 (profilin), Ara h 6 and Ara h 7 are first described in their structure deduced from the DNA sequence. Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to seed storage proteins, whereby Ara h 6 and 7 belong to the conglutin family. Ara h 3 is an isoform of Ara h 4 with 91% identity. An elegant way to overcome the expression problems of recombinant peanut allergens in E. coli was found. This opened the way to examining the question whether certain peanut allergens are associated with clin. symptoms and the severity of the clin. reactions. The fact that Ara h 6 was detected by the IgE of patients with shock symptoms and urticaria but not by the IgE of patients

with an isolated oral allergy syndrome may be an indication that Ara h 6 is a candidate for association with severe clin. reactions.

```
=> s l6 and kiwi
L42          0 L6 AND KIWI

=> s l6 and brazil nut
L43          0 L6 AND BRAZIL NUT

=> s l6 and potato
L44          0 L6 AND POTATO

=> s l44 and walnut
L45          0 L44 AND WALNUT

=> s l6 and castor bean
L46          0 L6 AND CASTOR BEAN

=> s l6 and nematode
L47          5 L6 AND NEMATODE

=> s l47 and pd<20000406
L48          0 L47 AND PD<20000406

=> s nematode antigen
L49        195 NEMATODE ANTIGEN

=> s l49 adn "Ani s1"
MISSING OPERATOR L49 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l149 and "Ani s1"
L149 NOT FOUND
The L-number entered could not be found.  To see the definition
of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s l49 and "Ani s1"
L50          0 L49 AND "ANI S1"

=> s anisakis simplex paramyosin
L51          2 ANISAKIS SIMPLEX PARAMYOSIN

=> dup remove l51
PROCESSING COMPLETED FOR L51
L52           1 DUP REMOVE L51 (1 DUPLICATE REMOVED)

=> s l52 and pd<20000406
L53          1 L52 AND PD<20000406

=> d l53 cbib abs

L53  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
2000:858441   Document No. 135:18479  Molecular cloning of paramyosin, a new
     allergen of Anisakis simplex.  Perez-Perez, Julian; Fernandez-Caldas,
     Enrique; Maranon, Francisco; Sastre, Joaquin; Bernal, Magdalena Lluch;
     Rodriguez, Julia; Bedate, Carlos Alonso (CBF LETI, SA. Research
     Laboratories Universidad Autonoma de Madrid, Madrid, E-28760, Spain).
     International Archives of Allergy and Immunology, 123(2), 120-129
     (English) 2000.  CODEN: IAAIEG.  ISSN: 1018-2438.  Publisher: S.
     Karger AG.
```

AB Anisakis simplex is a fish parasite that, when accidentally ingested by humans, may cause allergic reactions in sensitized individuals. The main objectives of the study were to: (1) construct a cDNA expression library of A. simplex; (2) identify clones producing specific IgE binding protein antigens, and (3) produce and purify the protein/s codified by the isolated clones produced in Escherichia coli. An expression cDNA library from the third stage larvae (L3) of A. simplex was constructed. This library was first screened with a rabbit anti A. simplex hyperimmune serum. The pos. clones, identified using the rabbit serum, were rescreened with a pool of human sera containing high titers of IgE antibodies against A. simplex. Two pos. clones were isolated carrying the genes which codify for paramyosin. The paramyosin protein was produced in E. coli and purified. The partial sequence of a second paramyosin gene was also identified. The frequency of specific IgE binding to the recombinant and native forms of paramyosin using the sera of 26 A. simplex-sensitive individuals was 23 and 88%, resp. Both paramyosins were able to inhibit 11% of the specific IgE binding to a total extract The authors describe the primary structure of a paramyosin of A. simplex. It can be considered as an allergen based on its IgE binding capacity. The authors suggest that the recombinant protein does not maintain the complete allergenic properties of the native paramyosin, considering its lower IgE binding capacity of the recombinant protein. However, both proteins have the same specific IgE inhibition capacity. The recombinant protein can be produced in large quantities in E. coli. The authors propose the term Ani s 2 for this allergen.

=> s l6 and ascaris suum antigen
L54 0 L6 AND ASCARIS SUUM ANTIGEN

=> s l6 and worm
L55 1 L6 AND WORM

=> d l55 cbib abs

L55 ANSWER 1 OF 1 MEDLINE on STN
1990337955 PubMed ID: 2199439. The hypoxanthine-guanine phosphoribosyltransferase of Schistosoma mansoni. Further characterization and gene expression in Escherichia coli. Yuan L; Craig S P; McKerrow J H; Wang C C. (Department of Pharmaceutical Chemistry, University of California, San Francisco 94143.) The Journal of biological chemistry, (1990 Aug 15) Vol. 265, No. 23, pp. 13528-32. Journal code: 2985121R. ISSN: 0021-9258. L-ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Due to the lack of de novo purine nucleotide biosynthesis, hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) is an essential enzyme in the human parasite Schistosoma mansoni for supplying guanine nucleotides and has been proposed as a potential target for antiparasitic chemotherapy. While the enzyme can be purified from adult schistosome **worms**, yields are too low to allow extensive structural and kinetic studies. We therefore cloned and sequenced the cDNA and gene encoding the schistosomal enzyme but were unable to positively identify the amino-terminal sequence of the enzyme from the DNA sequence. Knowledge of the exact amino terminus was necessary before accurate expression of active enzyme could be attempted. Therefore, we purified the HGPRTase from crude extracts of the adult **worms**. The purified enzyme has a subunit molecular mass of 26 kDa and an amino-terminal sequence of Met-Ser-Ser-Asn-Met. This sequence matched one of the potential initiation sites predicted from the cDNA and gene sequence. We next expressed the correct size cDNA of the S. mansoni HGPRTase in Escherichia coli using a vector that is regulated by a bacterial alkaline phosphatase promoter and uses an **E**.

coli signal peptide for secretion of expressed product into the periplasmic space. Using this expression system, some of the recombinant enzyme is secreted and found to have a correct amino terminus. That remaining in the cytoplasm has part of the signal peptide attached to the amino terminus. The recombinant schistosomal HGPRTase isolated from the **periplasm** of the transformed **E. coli** was purified and found to have kinetic and physical properties identical to those of the native enzyme.

```
=> s l6 and mosquito
L56          0 L6 AND MOSQUITO

=> s l6 and apyrase
L57          0 L6 AND APYRASE

=> s l6 and "Aed a2"
L58          0 L6 AND "AED A2"

=> s l6 and rubber
L59          0 L6 AND RUBBER

=> s l6 and flea
L60          0 L6 AND FLEA

=> s l6 and rubber
L61          0 L6 AND RUBBER

=> s l6 and human autoallergen
L62          0 L6 AND HUMAN AUTOALLERGEN

=> s human autoallergen
L63          1 HUMAN AUTOALLERGEN

=> d l63 cbib abs

L63  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2011 ACS on STN
2007:1052514   Document No. 147:384048  Modified vaccinia virus Ankara (MVA)
     encoding a recombinant allergen for the treatment of type I
     hypersensitivity in animals and humans.  Albrecht, Melanie; Sutter, Gerd;
     Suezer, Yasemin; Reese, Gerald; Vieths, Stefan; Staib, Caroline
     (Paul-Ehrlich-Institut Bundesamt fuer Sera und Impfstoffe, Germany).  Eur.
     Pat. Appl. EP 1835031 A1 20070919, 40pp.  DESIGNATED STATES: R:  AT, BE,
     BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LT,
     LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, AL, BA, HR, MK, YU.
     (English).  CODEN: EPXXDW.  APPLICATION: EP 2006-5164 20060314.
AB   The present invention relates to the use of a recombinant modified
     vaccinia virus Ankara (MVA) comprising a heterologous nucleic acid for the
     production of a medicament for the prevention and/or treatment of type I
     hypersensitivity in a living animal including humans.  The invention
     further relates to a recombinant modified vaccinia virus Ankara (MVA)
     comprising a heterologous nucleic acid, wherein the heterologous nucleic
     acid is incorporated into a non-essential region of the genome of the MVA,
     the heterologous nucleic acid is under the control of, e.g. a vaccinia
     virus-specific promoter and, the heterologous nucleic acid is selected
     from the group of nucleic acids encoding an allergen selected from the
     group of weed pollens, grass pollens, tree pollens, mites, animals, fungi,
     insects, rubber, worms, human autoallergens, and
     foods.

=>
```

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	63.60	63.83

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-4.35	-4.35

STN INTERNATIONAL LOGOFF AT 14:39:40 ON 23 FEB 2011